EFFECTS OF INDUSTRIAL CONTAMINATION ON CARRION-ASSOCIATED BEETLES

by

ANSLEY ELIZABETH DE LIMA SILVA

(Under the Direction of Kamal J. K. Gandhi and James C. Beasley)

ABSTRACT

Anthropogenic activities such as industrial contamination can have substantive impacts on biological communities. These impacts have been quantified for diverse assemblages of taxa, yet, to date there have been few studies that have investigated the effects of anthropogenic contaminants on decomposer communities in terrestrial ecosystems. In this research, I studied the effects of trace metals from coal combustion waste and radiocesium from nuclear effluents on communities of scavenger and predatory beetles associated with carrion decomposition, as well as the bioaccumulation of contaminants within these taxa at the Savannah River Site, South Carolina, U.S. Our results reveal variability in activity abundance (the number of individuals collected), species diversity, and species composition between contaminated and uncontaminated sites. Specifically, there was higher beetle activity abundance and diversity at contaminated locations as reflected in scavenger but not predatory beetles. Contaminated and uncontaminated sites had unique species compositions, however varying habitat conditions likely influenced these patterns. Specifically, habitat edges demonstrated unique species compositions compared to other trap locations. We observed higher levels of Arsenic (As), Selenium (Se), and Thallium (Tl) in scavenger beetles at a site contaminated with coal combustion waste, but higher levels of Chromium (Cr), Copper (Cu), and Nickel (Ni) at the uncontaminated site. Our study suggests that carrion-associated beetles are sentinel species for elucidating habitat quality and presence of anthropogenic contaminants.

INDEX WORDS: bioaccumulation, bioindication, carrion associated beetles, decomposition, industrial contamination, Savannah River Site

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DEDICATION

I dedicate this work to my parents, whom I love and respect.

Thank you both so much.

And to my partner, Rafael. Eu te agradeço.

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Anthropogenic contamination is a pervasive and detrimental impact that has serious consequences for biotic species, populations, and communities. Sources of industrial contamination include sewage sludge, pesticides, fertilizers, animal manure, coal combustion waste and radioactive materials from nuclear activities (Khan et al. 2008). In particular, coal combustion waste from power production facilities is a global issue that has been widely recognized for its negative impacts to biotic communities (Cherry and Guthrie 1977, Hopkins et al. 1997, Rowe et al. 2002, Fletcher et al. 2014) The U.S. alones generates approximately 100 million metric tons of coal combustion waste annually that is stored in 735 surface impoundments (US-EPA 2015). Coal combustion waste is of particular concern due to the presence of concentrated trace elements including Arsenic (As), Cadmium (Cd), Chromium (Cr), Copper (Cu), Selenium (Se), Strontium (Sr), and Zinc (Zn) that can bioaccumulate in food webs (Rowe et al. 2002). Accidents involving coal combustion waste, such as coal ash spill in the Emory River of Tennessee in 2008, can have long-term effects on ecological system (Ruhl et al. 2012).

Another source of contamination of great concern is radioactive materials from nuclear waste. Human activities have increased the magnitude of ionizing radiation in the environment through nuclear weapons testing and accidents (Chernobyl and

Fukushima). For example, large amounts of radioactive materials such as radiocesium (^{137}Cs) were released into the environment following the Chernobyl nuclear reactor disaster in the Ukraine (formerly in Russia) in 1986 (Dreicer et al. 1996). In the U.S., radioactive waste from plutonium production at the Harford Site in southeastern Washington contaminated the soil and groundwater (US-DOE 2016). Events such as these can have profound and long-lasting effects on biota and ecological systems.

Bioindication, the use of living organisms to monitor the impact of a disturbance, is a valuable alternative to direct, abiotic monitoring. Moreover, organisms across taxa, although different in their ecology, might demonstrate strong congruence in their response and recovery processes when experiencing anthropogenic disturbances (Aubin et al. 2013). Numerous studies have shown the importance of invertebrates as biodiversity indicators due to their potential to act as surrogates for the larger community (Mietelski et al. 2003, Rainio and Niemela 2003, Aubin et al. 2013, Ayabe et al. 2014) and value in assessing the degree of contamination (Gongalsky 2003, Babin-Fenske and Anand 2011).

In particular, the use of insects as biodiversity indicators offers advantages because invertebrate provide insight to habitat quality, are relatively easy to sample, and demonstrate large numbers of individual for obtaining adequate sample sizes. For example, many insect taxa express varying degrees of sensitivity to an environmental disturbance and we can select taxa that would best reflect the impact of a certain disturbance. Further, insect orders encompass all functional groups—from primary consumers to top predators, and decomposers. Since ecosystem functions are linked to functional groups, groups of taxa can be used to monitor the desired ecosystem function

(Duffy et al. 2007, Aubin et al. 2013). Trophic food webs directly influence ecosystem functions, such as energy flow and nutrient cycling (Duffy et al. 2007, Estes et al. 2011) and decomposers play a major role in these processes because they feed at every level of the food web. Invertebrates are unrivaled in their contribution to ecosystem functions and processes, therefore the assessment of their biodiversity is an important field in ecology (Kim 1993).

Invertebrates are utilized as bioindicators of environmental changes and habitat quality because their responses can generally be measured across a gradient of levels of organization, ranging from the individual to community (Hodkinson and Jackson 2005). Short-term responses may be best measured at the individual level such as physiology and behavior. For example, individual-level responses such respiration rates, morphological deformities and bioaccumulation of toxins generally are useful for assessing short-term responses to anthropogenic disturbances. Population (multiple individuals of one species) level responses such as changing densities of the bioindicator of interest, which can be useful for both short and long-term studies. Community level responses integrate multiple population responses and, although complex, this level of interest is important for gaining insight to long-term impacts of anthropogenic disturbance. Hence, the level of organization, from individual to community, is an important choice in investigating environmental conditions and ecosystem functions (Hodkinson and Jackson 2005).

Decomposition is a fundamental ecosystem function related to energy and nutrient fluxes in terrestrial ecosystems (Putman 1978, Towne 2000, Carter et al. 2007, Parmenter and MacMahon 2009). Specifically, decomposing vertebrates represent an intense resource pulse for terrestrial systems, which results in fierce competition among bacteria, invertebrates, and vertebrates to assimilate the nutrients. Thus, carrion is an ephemeral and unpredictable resource that produces massed heterotrophic activity in localized areas. Although ephemeral, this type of resource patch can have long-lived effects on the local soil (Towne 2000) and the influence of this localized flux of nutrients can positively affect local biodiversity (Carter et al. 2007).

Of the organisms associated with carrion, invertebrates play a particularly critical role in the decomposition and recycling of nutrients within food (Payne 1965, 1968, Devault et al. 2003). Moreover, some invertebrates in the Order Coleoptera are not only associated with decomposition, but also are known bioindicators of ecosystem health (Gibbs and Stanton 2001, Arellano 2003, Mietelski et al. 2003, Rainio and Niemela 2003, Ulyshen and Hanula 2004). Further, their role in decomposition is important to food-web architecture, and perhaps increasingly important due to trophic downgrading and the disappearance of top predators (Estes et al. 2001, Beasley et al. 2012). However, carrion beetles (Silphidae) are highly impacted by anthropogenic disturbances (Creighton et al. 2001, Gibbs and Stanton 2001). Silphidae beetles have specific breeding habitat requirements involving carrion and thus, depend on carrion for survival (Majka 2011). The silphid *Nicrophorus americanus* (Olivier) of eastern North American has been listed as endangered by the U.S. Fish and Wildlife Service (1991), *N. germanicus* (L.) of Europe is also listed as endangered, and *N. marginatus* (F.) is in decline. In fact, recent years have shown a world-wide decline in large-bodied beetles in general (Trumbo and Block 2000). Most species of *Nicrophorus* are sensitive to disturbance, (Lomolino and Creighton 1996, Gibbs and Stanton 2001, Creighton 2009) especially deforestation, which results in warmer soils (Trumbo and Bloch 2000). This genus can be characterized as close-canopy species that require soft soils for burying carcasses. The decline of some species of silphids could also be the result of the reduced presence of small mammals—the primary food base of silphids—in disturbed habitats (Creighton 2009). Despite the importance of invertebrate scavengers to food webs, little is known about how specific disturbances —e.g., contamination by radioactive pollution and metals—affect their assemblages. Furthermore, to our knowledge, no inventories or carrion associated beetles have been documented at our study-sites in South Carolina, U.S.A.; nor have these organisms been analyzed for trace element and radiocesium contamination. Given their critical role in ecosystem function and limited data on the effects of anthropogenic disturbance on communities and individuals of carrion-beetles

1.2 Thesis Objectives

This study utilized Coleoptera as bioindicators of changing chemical environment in regards to trace metal and radionuclide contamination and as a comparison of habitat quality. While carrion and dung beetles are not charismatic in terms of public perception, they have been shown to be good bioindicators of anthropogenic disturbance (Howden and Nealis 1975; Klein 1989, Nummelin and Hanski 1989, Halffter et al. 1992, Hill 1996, Davis and Sutton 1998, Estrada et al. 1998, Davis et al. 2001, Estrada and Coates-Estrada 2002). To our knowledge, this is the first inventory of carrion associated beetles at the Savannah River Site in the coastal plain of South Carolina.

Our main research objectives were as follows: 1) to assess the effects of two types of contamination (coal fly-ash and radiocesium) on carrion-associated beetle assemblages; and 2) to determine if individual carrion insects that are active closer to contaminated sites bioaccumulate elevated levels of metals and radioactivity in their bodies compared to individuals collected at uncontaminated reference sites.

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CHAPTER 2

EFFECTS OF TRACE ELEMENT AND RADIOCESIUM CONTAMINATION ON ASSEMBLAGES OF CARRION-ASSOCIATED BEETLES

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Abstract

Energy production systems such as nuclear reactors and coal-burning power plants produce substantial waste containing a multitude of contaminants including radionuclides, trace elements, and heavy metals. These contaminants can have significant, yet subtle effects on biotic communities. To date, most of the existing literature has focused on effects of anthropogenic contamination on invertebrates in aquatic environments, while little is known about the effects of contamination on terrestrial invertebrate communities. Since biotic communities can influence ecosystem functions, we investigated the influence of trace element and radionuclide contamination on assemblages of carrion-associated beetles (Coleoptera: Scarabaeidae and Silphidae) that comprise critical decomposition taxa. Samples were collected from each of three contaminated and uncontaminated sites at the Savannah River Site in South Carolina to compare beetle activity abundance (the number of individual beetles captured) and species diversity (using rarefaction diversity index) among sites and along a habitat gradient from the water source. During this study we collected 17,800 carrion-associated beetle individuals representing 112 species in nine families, which we classified into two groups as scavenger and predatory beetles. Beetle activity abundance and species diversity was generally higher at contaminated than uncontaminated sites, perhaps reflecting greater habitat disturbances. These trends were likely driven by scavenger species that showed similar patterns, whereas patterns of activity abundance and species diversity were variable for predatory beetles. Species compositions of contaminated and uncontaminated sites were generally distinct, however habitat edges appear to affect beetle assemblages as well. Overall, our study documents impacts of various chemical and radioactive contaminations as well as habitat disturbances on assemblages of ecologically important invertebrate taxa in southeastern forest stands.

INDEX WORDS: assemblage, biodiversity, carrion-associated beetles, coal combustion waste, radionuclide, Savannah River Site

2.1 Introduction

A suite of human activities increasingly affects ecosystems across the world. Anthropogenic disturbances vary in type, extent, and severity with implications for biotic communities and ecosystem processes (Addessi 1994, Simmons et al. 2008, Butt et al. 2013). Contamination caused by energy producing facilities is of particular concern because of the expansive range of unintended and non-target effects. Contaminants such as radionuclides and trace elements accumulate in soils can influence the composition, structure, and function of ecosystems (Sutherland 2000, Emmanuel et al. 2002, Miralles et al. 2004). However, due to the complexity of ecosystem responses to contamination, studies investigating the fate, transport, and effects of anthropogenic contaminants on ecosystems remain an important area of research.

Most ecotoxicology studies have focused on the individual or population level, but there is also a need for understanding how pollutants affect communities, since studies at the individual or population scale may overlook broader processes impacting community or ecosystem dynamics (Gall et al. 2015). For example, studies at the individual level may be useful for determining short-term responses of organisms to particular contaminants, whereas community level studies may elucidate long-term and multi species-level consequences of environmental degradation (Hodkinson and Jackson 2005). In particular, studies focused on targeted guilds, such as scavenger or predatory species, have the highest discriminatory power to investigate disturbance gradients (Basset et al. 2002, Schipper et al. 2010).

Indicator species or bioindicators, are species that are ecological indicators of habitat conditions and quality (McGeoch 1998). Bioindicators are helpful tools in monitoring environmental gradients to anthropogenic disturbances such as point-source pollution. Decreased abundance and species richness of aquatic invertebrates due to chemical contamination has been well-documented (e.g., Cherry et al. 1979, Scullion et al. 1980, Duchrow 1982, VanHassel et al. 1984) and thus historically, aquatic invertebrates have been used frequently for monitoring water quality (Rosenburg et al. 1986, Hodkinson and Jackson 2005). Although routine methods for measuring the effects of pollution on aquatic invertebrate communities exist (Karr 1991), there is not yet a consensus on taxa for terrestrial invertebrates due to highly diverse life-histories (Basset et al. 2002). Nonetheless, terrestrial invertebrates are valuable indicators of ecological conditions. In particular, terrestrial invertebrates that are species-rich, abundant, widespread, and serve critical functional roles within ecosystems likely represent useful bioindicator taxa.

2.2 Research Objective

Carrion beetles (such as those from the genus *Nicrophorus*) are known bioindicators of forest disturbances and have been shown to decline in both numbers and diversity with an increase in disturbance (Gibbs and Stanton 2001). Given their critical role in nutrient cycling and sensitivity to disturbance, our objective was to use carrion-associated beetles as biodindicator taxa to test the hypothesis that assemblages of carrion-associated beetles are negatively impacted by anthropogenic contamination from power production facilities. More specifically, we investigated shifts in scavenger and predatory beetle assemblages and activity abundance (number of individuals captured) along a gradient from the water's edge (0-300 m away from the edge) as an effect of habitat, and at contaminated compared to uncontaminated water bodies.

2.3 Methods

2.3.1 Study Area

This study was conducted within the U.S. Department of Energy's (DOE) Savannah River Site (SRS) located in Aiken, Allendale, and Barnwell Counties, South Carolina, U.S. The SRS is located halfway between the mountains and the coast in a region broadly referred to as the Sand Hills. The SRS encompasses a ~780 km^2 area at elevations ranging from 30-120 m above sea level. Soil types vary across the SRS due to many factors including differences in topography and parent material, climate, and soil degradation resulting from agricultural practices. The main soil series found at the SRS include Ailey sand, Blanton sand, Chastain clay, Dorovan muck, Dothan sand, Fluvaquents, Fuguay sand, Lakeland sand, Orangeburg loamy sand, Pickney sand, Rembert sandy loam, Troup sand, Udorthents, Vaucluse-Alley complex, and Wagrum sand (Soil Conservation Service 1990). The average winter temperature is 8.61 °C with an average minimum winter temperature of 2.61 °C. The average summer temperature is 26.53 °C with an average maximum of 32.28 °C (Soil Conservation Service 1990).

Pine forest, longleaf pine savannah, sandhill woodlands, and isolated wetland depressions are present on the SRS. The dominant forest cover-types include loblolly (*P. taeda* L.) and longleaf (*P. palustris* Mill) pines as well as oak-hickory (*Quercus* and *Carya*) and tupelo-cypress (*Nyssa* and *Cupressus*). Dominant understory vegetation includes herbaceous species of forbs and grasses, and woody species of vines and shrubs.

The SRS contains hundreds of Carolina bays and other isolated wetlands, which have been left mainly undisturbed since the area was restricted from public access in 1950 (White et al. 2000, DeVault et al. 2003). Although approximately 81% of the land is forested and successful bioremediation efforts exist, there is a history of environmental contamination at the SRS.

SRS was acquired in 1950 by the former U.S. Atomic Energy Commission for nuclear weapons materials production (White and Gaines 2000). Five nuclear reactors and associated facilities were constructed and the production of basic nuclear materials such as plutonium-237 and tritium ran until 1988 at the end of the Cold War. All nuclear reactors and powerhouses have since been decommissioned. Current operations at the SRS include nuclear materials processing and stockpile maintenance, tritium extraction and purification, sanitary wastewater treatment, and landfill facilities. SRS is the nation's first National Environmental Research Park; a place where studies can be conducted to quantify the effects of human impacts on the environment. This site provides unique opportunities for research due to isolated areas of elevated contaminant levels, primarily at select aquatic systems. Although radiocesium has been the main contaminant of concern at many locations within the SRS, other contaminants such as heavy metals and trace elements also exist in localized areas. Given the unique juxtaposition of contaminated and uncontaminated sites and the variable composition of contaminants present, the SRS is an ideal location for investigating the impacts of anthropogenic contaminants on biotic systems.

2.3.2 Sampling Sites

We selected six locations within the SRS for collecting beetles (Fig. 2.1). Three of these locations represented contaminated sites where each site had a different contaminant input: 1) radiocesium (Pond A); 2) coal-fly ash (Ash Basin); and 3) coal run-off (Acid Pond) (see below for site descriptions). The remaining three locations were control sites that have not been directly impacted by coal waste products or radiocesium. These control sites were selected to approximate the habitat conditions present at the contaminated sites. At the Ash Basin, overstory and understory cover was ~8% and 14% respectively at the water's edge (0 m) and 73% and 49% at further distances (100-300 m) from the water's edge. Similar conditions were present at the Acid Pond where overstory and understory cover was ~0% and 34%, respectively at the water's edge and 60% and 50% away from the water's edge. At Pond A, overstory and understory cover was ~70% and 50%, respectively at the water's edge and 60% and 25% away from the water. Both Steel Creek Bay and Pond T had overstory and understory cover that was ~70% and 60%, respectively adjacent and away from the water's edge. Overstory and understory cover at Thunder Bay was ~18% and 40%, respectively at the water's edge and 63% and 41% away from the water's edge.

When coal is burned, naturally occurring trace amounts of metals become concentrated residuals in ash. These combustion residuals can contain toxic levels of Arsenic (As), Cadmium (Cd), Chromium (Cr), Copper (Cu), Selenium (Se), and Strontium (Sr) (Rowe et al. 2002). At SRS, D-Area was previously the location of a heavy water facility, which produced deuterium oxide, and a coal-fired powerhouse. Coal ash produced at this facility was stored in several nearby surface impoundments and as a result of these activities contaminants such as heavy metals and trace elements became concentrated at coal-fly ash basins and a coal pile run-off basin (Brofft et al. 2002). Notably, these impoundments are accessible and utilized by the wildlife in this region and adverse effects and bioaccumulation of trace elements has been previously documented (e.g., Hopkins et al. 1997, Rowe et al. 2002). The coal pile run-off basin in D-Area is adjacent to a natural wetland that has been impacted to extent that it no longer supports vegetation (Carlson 1990). Both the coal ash basins and the coal pile run-off basin at D-area were selected as sampling sites for this research.

Construction for nuclear facilities at the SRS began in 1951 and operations of these facilities continued until 1988. During 1954-1964, R-reactor released approximately 8.2 X 10^{12} Bq of radiocesium into the surrounding drainage system from spent fuel rods and target slugs that were not properly sealed (Carlton et al. 1992). Radiocesium leached into a system of reservoirs via a cooling water outflow (R-canal) including Pond A (5.2 ha and < 1 m deep), a cooling impoundment located along R-canal where deposition from R-reactor first occurred (Abraham et al. 2000). As of 1996, it has been estimated that Pond A has a total ^{137}Cs inventory of 4.1 X 10 ^{10}Bq (Abraham et al. 2000). Given the extent and history of ^{137}Cs contamination at Pond A, this site was selected as a radionuclide contaminated sampling location for this research.

Three additional (control) sites were selected for this study, each of which was paired with a specific contaminated site based on similarity in size and habitat type: 1) T Pond (paired with the Ash Basin); 2) Steel Creek Bay (paired with Acid Pond); and 3) Thunder Bay (paired with Pond A). Control sites had no known inputs of contaminants due to SRS industrial activities. Because the contaminant composition and types differed for each site, we considered the three site-pairs to be individual treatments in this study.

2.3.3 Beetle Collection

Beetles were collected using pitfall traps consisting of a 5.7 L basin placed inside a larger 26.5 L basin filled with approximately 5 cm of propylene glycol (Fig. 2.2). The 26.5 L basin was placed in the soil with the rim of the basin flush with the topsoil to also allow capture of beetles travelling along the ground. Drainage holes were made on all sides of the 26.5 L basin to avoid flooding during rain (Fig. 2.2). For carrion bait, we used medium-sized rabbit carcasses (0.494-2.476 Kg) obtained from Rodent Pro, a commercial breeder. Rabbits were euthanized via cervical dislocation and frozen by Rodent Pro. Prior to deployment in the field, carcasses were thawed indoors to ambient temperature and transported to the field in a sealed container to avoid invertebrate colonization prior to deployment in the field. We placed carrion within the 5.7 L basin on a substratum of 5 cm sawdust. To protect the bait from vertebrate scavengers, an empty Tomahawk Livetrap (Tomahawk Live Trap Co., Tomahawk, Wisconsin) was set over the basins and secured by four rebar stakes. The trap was designed to specifically capture beetles associated with vertebrate carrion.

We sampled each site pair simultaneously during August 2014 or June-July 2015. In 2014, we established 10 transects >75 m apart each at T Pond (control) and the D-Area Ash Basin (contaminated). Along each transect we placed a sampling location directly adjacent to the edge of the water (0 m) with additional sampling locations 100, 200, and 300 m away from the water's edge. Due to high capture success in 2014, we reduced the number of transects to six at each uncontaminated and contaminated site (Steel Creek Bay and Acid Pond; Thunder Bay and Pond A) in 2015, with the same trap spacing within transects. Hence, a total of 174 traps were operated for the entire two-year study.

All traps were open for seven days, a period sufficient for advanced decomposition of the carrion bait (*personal observations*). Upon entering the trap, captured beetles were preserved in the propylene glycol barricade surrounding the carrion until they were collected. Samples were frozen until cleaned of debris and rinsed, and transferred to 70% ethanol. Adult beetles were identified using available keys and species descriptions (Ciegler 2000, Arnett et al. 2001, 2002, Pearson et al. 2006). A reference collection was deposited at the Georgia Museum of Natural History, University of Georgia, Athens, Georgia, US.

2.3.4 Data Analyses

Given that the type of contamination differed among the three site-pairs, we analyzed each pair individually. Further, we separated species into three groupings as: 1) all beetle species combined; 2) scavenger beetles; and 3) predatory beetles. We distinguished scavenger beetles as those that specifically consume vertebrate carrion in the adult stage, including the families Dermestidae, Geotrupidae, Scarabaeidae, Silphidae, and Trogidae. Predatory beetles were distinguished as those that are attracted to carrion to prey on other invertebrates, including the families Carabidae, Cicindelidae, Histeridae, and Staphylinidae.

For each trap location we counted the total number of Coleoptera, hereafter referred to as activity abundance. We used multiple regression models to examine whether the main effects of treatment (uncontaminated or contaminated) and distance from water source (0-300 m), and their interaction had an effect on activity abundance of beetles. Distance away from water was analyzed on a case-by-case basis for each of the three site-pairs and three species groupings. After visualizing the data with scatter plots, distance was treated either as 1) a continuous variable when displaying a linear relationship to activity abundance; 2) a categorical variable (with levels of 0, 100, 200, and 300 m) when there was no linear relationship; or 3) as binomial variable (with levels 0 m and > 0 m) when there was a linear relationship from 0 to 100 m but stabilized at 100-300 m. After examining the data for violations of regression assumptions (homoscedacity and normality of residuals), we transformed activity abundance to the natural log scale. Regression analyses were performed using R statistical software (package: lattice) (R Development Core Team 2008).

We constructed species accumulation curves using individual-based rarefaction diversity indices for each of the three site-pairs and species groupings. These curves allow for comparisons among sites because they standardize sampling effort by using an algorithm to resample from the collection (Sanders 1968, Hurlbert 1971, Magurran 2004). These descriptive curves indicate the nature of species accumulation e.g., steeper and longer slopes suggest faster and greater species accumulation at a specific site. Further, we assessed differences in species diversity between sites and locations using the lowest number of individuals accumulated for each site comparison.

To elucidate differences in species composition between uncontaminated and contaminated sites, we used Similarity Profile routines (SIMPROF, Clark and Warlock 2001) to conduct permutation tests of hierarchical cluster analysis. From these analyses, we created dendrograms that range from small clusters of similar items to larger clusters of expanding dissimilar items. One thousand expected similarity profiles and 999 observed similarity profiles were generated using the distance measure Bray-Curtis as the clustering algorithm and average linkage which emphasizes the central tendency of the data and is less sensitive to outliers than other linkage methods (Bray 1957, Clark 1993, McCune 2002). We also used indicator species analysis for each site combination and distance to determine if specific species were more associated with certain locations (Dufrene and Legendre 1997). Dendrograms and indicator species analysis were performed using R statistical software (packages: vegan, clustsig and indicspecies) (R Development Core Team 2008).

2.4 Results

Overall, we collected 17,800 individual carrion-associated beetles from 112 species in 9 families (Appendices A, B, and C). Scavenger and predatory beetles composed 11,035 and 6,765 individuals and 54 and 58 species, respectively. The most numerous species were *Euspilotus assimilis* (Paykull) (4,556), *Deltochilum gibbosum* (F.) (2,823), *Ateuchus lecontei* (Harold) (1,794), *Onthophagus hecate hecate* (Panzer) (1, 448), and *Nicrophorus orbicollis* (Say) (1,227).

2.4.1 Beetle Activity Abundance

Results from comparisons of Ash Basin (coal-fly ash) and Pond T (paired reference site) revealed that total beetle activity abundance was higher at the contaminated site, but only at distances greater than 0 m away from the water's edge

(Table 2.1). This pattern was driven by the activity abundance of scavengers that exhibited similar trends; no such trends were found for predators. Comparisons of total beetle and scavenger activity abundance between Acid Pond (coal pile run-off) and Steel Creek Bay (paired reference site) revealed that both metrics were higher further away from water regardless of site location. No site-specific or distance class trends were observed for predatory beetles. At Pond A (radiocesium) and Thunder Bay (paired reference site), total beetle and scavenger activity abundance were higher at the contaminated site. There was an increase in activity abundance of both total beetle and scavengers with increasing distance at Thunder Bay. However we found a decrease in activity abundance of both total beetle and scavengers with increasing distance at the contaminated site (Table 2.1). There were no trends for predatory beetles in activity abundance at Pond A and Thunder Bay.

2.4.3 Species Accumulation Curves

For the site-pair Ash Basin/Pond T, we observed higher species accumulation at the contaminated than uncontaminated site (Fig. 2.3 A). Similarly, higher species accumulation was observed at the 300 m trap except for predatory beetles that had at it 100 and 200 m traps at the contaminated sites (Figs. 2.4 A-B). For the site-pair Acid Pond/Steel Creek Bay, the contaminated site also accumulated more species with increasing number of beetles (Figs. 2.5 A, B), except for predatory beetles (Fig. 2.5 C). The 200 m trap seemed to accumulate the greatest species for this pair (Fig. 2.6). For the site-pair comparison Pond A/Thunder Bay, the contaminated site also accumulated more species, except for predatory beetles (Fig. 2.7). The 0 m traps at the contaminated site and 300 m traps at the uncontaminated sites accumulated the most species when considering all beetles combined, whereas the 0 m trap at the contaminated site accumulated the most scavenging beetles and the 100 m trap at the uncontaminated site had the most predatory beetles (Fig. 2.8).

2.4.4 Species Composition

Overall, 0 m traps tended to have unique species composition, and except for few instances, contaminated and uncontaminated sites had different beetle composition. .Specifically, for the Ash Basin/Pond T site-pair, the 0 m traps at both the sites clustered separately while the uncontaminated and contaminated site traps tended to cluster together for all the pooled species (Fig. 2.9 A). Similarly, for scavenging and predatory beetles, 0 m traps at the contaminated site were distinct (Fig 2.9 B-C).

For the Acid Pond/Steel Creek Bay site-pair, 0 m at the uncontaminated site, 0 and 100 m at the contaminated site, and the rest of the trap locations clustered out separately for all pooled species (Fig. 2.10 A). The, 0 m trap had distinct species composition of scavenging beetles and those within contaminated and uncontaminated sites clustered together (Fig. 2.10 B). No clear patterns were seen for predatory beetles (Fig. 2.10 C).

At the Pond A/Thunder Bay site-pair, 0 m traps at the uncontaminated and contaminated sites, and 100 m trap at the contaminated site clustered out separately for all pooled species and scavenging beetles (Fig 2.11 A-B). For the predatory beetles, 0 m traps had distinct species composition with the traps within contaminated and uncontaminated sites clustering together (Fig 2.11 C).

Indicator Species Analysis determined that indicator species were generally distinct as based on sites and locations. *Creophilus maxillosus* (L.) was an indicator species at the Ash Basin (Table 2.2). At distances > 0 m from water's edge, Ash Basin and Pond T sites, respectively, had five [*Copris minutus* (Drury), *D. gibbosum, N. orbicollis, Omorgus monachus* (Herbst), and *Onthophagus striatulus* (Beauvois)] and three [*C. minutus, D. gibbosum*, and *Trox foveicolli* (Harold)] indicator species (Table 2.2). *Onthophagus hecate hecate* (Panzer) was the indicator species for both Acid Pond and Pond A along with *N. orbicollis* at distances >0 m away from water's edge at both sites. Four indicator species [*Canthon chalites* (Haldenman), *Hister coenosus* (Erichson), *N. orbicollis*, and *O. hecate hecate*)] were present at Steel Creek Bay. *Onthophagus h. hecate* was also an indicator species at the two most proximal distances to the water's edge, and at the Thunder Bay site at the water's edge. Additionally, at Thunder Bay, *D. gibbosum*, *Nicrophorus tormentosus* (Weber) were indicators at > 0 m away from the water's edge.

2.5. Discussion

In this study we investigated changes in the activity abundance (number of individuals captured), species diversity, and species composition of carrion-associated beetles along a distance gradient from aquatic habitats and as a function of habitat contamination. Our study revealed variability and confounding trends in species diversity among sampling sites suggesting site-specific habitat or other environmental attributes are likely drivers of Coleopteran assemblages (Gibbs and Stanton 2001). However, in general we observed higher activity abundance and diversity at contaminated sites compared to uncontaminated reference sites. This pattern was particularly pronounced for scavenging beetles, whereas weak or no trends were observed for predatory beetles. Compared to reference sites, contaminated sites generally supported as high or higher numbers of captured beetles and greater diversity. However, further delineation of our data into scavenger and predatory species revealed inter-guild differences in these trends. Activity abundance was higher for scavenger beetles and lower for predatory beetles at contaminated than uncontaminated sites. Diversity for both groups was inconsistent across the site-pairs. In most cases, regardless of whether a site was contaminated or uncontaminated, we observed a unique composition of beetles (scavengers and predators) adjacent to the water's edge compared to those captured within forest interiors.

Previous studies have shown that initial impacts from coal contaminants on invertebrate communities are characterized by decreases in species richness and total abundance due to increased mortality and/or emigration (Harper et al. 2004). Species diversity of carabid beetles decreased with increasing heavy metal concentration in southern Poland, which was attributed to autumn breeders being more sensitive to pollution than spring breeder (Skalski et al. 2010). However, organisms can demonstrate increased diversity and numbers following a disturbance due to creation of new niches and habitats (Roxburgh et al. 2004). For example, dung beetles have demonstrated high abundance and species richness along a gradient of habitat fragmentation (Quintero and Roslin 2005). Carabid beetle diversity typically increases after forest harvesting activities, which is attributed to invasion by open-habitat species (Koivula et al. 2002). It is possible that our trends may also reflect the Intermediate Disturbance Hypothesis, which is a non-linear trend for increased species activity with disturbance regimes (Wilson 1990). Differences in species composition amongst the scavenger and predatory beetles may be due to differing suitability in microhabitats along the environmental gradients in our study, and that we may have sampled only a subset of these microhabitats.

Species compositions of contaminated (Ash Basin and Acid Pond) and uncontaminated sites were generally distinct especially as related to scavenging beetles. This suggests species may vary in their sensitivity to contaminants and that heavy metals from coal processing plants may be adversely affecting certain scavenging beetle species leading to altered assemblages. For example, *Melancanthin bispinatus* (Robinson), three species of *Phyllophaga* and *Phileurus* were absent at the Ash Basin and Acid Pond, but present at control sites. Other studies have indicated similar effects of heavy metals on mayflies in Colorado, U.S. (Clements et al. 2000), ants in Poland (Grzes 2009), and wild bees in Poland and the U.K. (Moron et al. 2011). Such differences could be due to differences in habitat structure, but also to the types and abundance of vertebrate prey, and species-specific impacts of pollution at the two treatment sites.

Although chemical disturbances might be partially contributing to the distribution patterns of beetles observed in this study, heterogeneity in forest cover is also known to influence the richness and abundance of beetles and likely contributed to our observations (Howden and Nealis 1975, Klein 1989). Not only do surface impoundments created to store coal combustion waste create chemical disturbances, but most impoundments result in physical disturbances to the landscape, such as abrupt landscape edges, decreased overstory, and soil compaction - all of which also can have a profound impact on community composition. For example at the Ash Basin and Acid pond sites habitat characteristics were drastically different adjacent to the water's edge where there was little to no overstory compared to other sampling locations at distances further way from the actual basins. Additionally, habitat at the radiocesium site, Pond A was relatively undisturbed at the water's edge yet surprisingly activity abundance decreased with increasing distance from the contaminated water source. This pattern likely reflects underlying differences in habitat composition at Pond A along some of our sampling transects, which were characterized by an abrupt change from mixed hardwood to loblolly stands with less overstory. Such habitat change exemplifies the role of habitat in regulating beetle assemblages.

Carrion-associated beetles play an integral role in decomposition of carrion and cycling of nutrients within food webs. Although these organisms are bioindicators of environmental change and habitat quality, there is less research investigating their responses to environmental contamination (Ermakov 2012). Our study revealed variability in the responses of carrion-associated beetles among contaminated and uncontaminated sites, likely influenced by heterogeneity in habitat attributes both among and within sites. Nonetheless, despite the presence of contaminants and physical degradation of habitat associated with all of our contaminant sites, both scavenging and predatory beetles were present in all 162 traps in this study and there was never a case when a trap didn't have multiple species of carrion-associated beetles. Chemical contamination, deforestation, abrupt habitat changes, and relatively undisturbed habitat at SRS may have created a dynamic landscape with variability in species abundance activity abundance (number of individuals captured), species diversity, and species composition of carrion-associated beetles.

Overall, our unique and first study on effects of such chemical contamination on carrion-associated beetles revealed increased abundance at contaminated sites. Although species compositions of contaminated and uncontaminated sites were generally distinct, habitat edges likely influenced beetle assemblages as well. Unique composition of carrion-associated beetles was generally found adjacent to bodies of water. Chemical contamination and abrupt habitat changes at SRS creates a dynamic landscape that creates variability in species abundance activity abundance (number of individuals captured), species diversity, and species composition of carrion-associated beetles. Long-term monitoring of multiple trophic levels at these sites may be necessary to assist with full-scale ecological recovery of contaminated areas in these southeastern forests.

2.6 Acknowledgments

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Table 2.1 Regression coefficients of simple and multiple linear models for activity abundance of carrion-associated beetle assemblages collected at the Savannah River Site in Aiken, South Carolina, USA during the summers of 2013-2014.

Site-Pair	Taxa	Variable	Estimate	SE	T-stat	R^2	F-stat	Odds Ratio	P-value
AB/ST	Total	Intercept	4.0055	0.1864	21.486	0.1645	$5.921_{3, 72}$	54.899	<0.001
		Treatment	-0.2413	0.2636	-0.915	-	-	0.786	NS
		Distance[a]	0.1612	0.2153	0.749	-	-	1.175	NS
		T*D	0.6654	0.3073	2.165	-	-	1.945	0.034
	Scavenger	Intercept	2.7955	0.1792	15.599	0.3708	$15.73_{3, 72}$	16.371	0.001
		Treatment	0.1490	0.2534	0.588	-	-	1.161	NS
		Distance[a]	0.3754	0.2069	1.814	-	-	1.456	NS
		T*D	0.6340	0.2954	2.146	-	-	1.885	0.035
	Predatory	Intercept	3.545	0.2720	12.919	-0.0179	$0.8168_{7, 68}$	34.640	<0.001
		Treatment	-0.3815	0.3847	-0.992	-	-	0.683	NS
		100m	0.3505	0.3847	0.911	-	-	1.420	NS
		200m	-0.2652	0.3847	-0.689	-	-	0.767	NS
		300m	0.1182	0.3847	0.307	-	-	1.125	NS
		T*100m	0.1365	0.5516	0.247	-	-	1.146	NS
		T*200m	0.7387	0.5516	1.339	-	-	2.093	NS
		T*300m	0.5350	0.5608	0.954	-	-	1.707	NS
AP/ST	Total	Intercept	4.5306	0.1630	27.793	0.0848	$5.264_{1, 45}$	92.821	0.001
		Distance[b]	0.2002	0.0873	2.294	-	-	1.222	0.027
	Scavenger	Intercept	3.9023	0.2000	19.516	0.2104	$13.26_{1, 45}$	49.516	<0.001
		Distance[a]	0.8438	0.2317	3.641	-	-	2.325	0.001
	Predatory	Intercept	3.388	0.4563	7.425	0.0621	$1.435_{7, 39}$	29.607	<0.001
		Treatment	-0.4837	0.6453	-0.750	-	-	0.616	NS
		100m	0.3668	0.6453	0.568	-	-	1.443	NS
		200m	-0.3047	0.6768	-0.450	-	-	0.737	NS
		300m	-0.5728	0.6453	-0.888	-	-	0.564	NS
		T*100m	-1.1519	0.9126	-1.262	-	-	0.316	NS
		T*200m	1.1588	0.9351	1.239	-	-	3.186	NS

		T*300m	1.0640	0.9126	1.166	-	-	2.898	NS
PA/TB	Total	Intercept	3.6291	0.3026	11.993	0.1076	$2.528_{3, 35}$	37.679	<0.001
		Treatment	1.1673	0.4790	2.437	-	-	3.213	0.020
		Distance[b]	0.3784	0.1617	2.339	-	-	1.460	0.025
		T*D	-0.5896	0.2568	-2.296	-	-	0.555	0.028
	Scavenger	Intercept	3.0455	0.2649	11.498	0.2628	$5.515_{3, 35}$	21.021	<0.001
		Treatment	1.6379	0.4193	3.906	-	-	5.144	<0.001
		Distance[b]	0.4176	0.1416	2.949	-	-	1.518	0.006
		T*D	-0.7472	0.2248	-3.324	-	-	0.474	0.002
	Predatory	Intercept	2.4565	0.4517	5.439	-0.0080	$0.8992_{3, 35}$	11.664	<0.001
		Treatment	0.1511	0.7150	0.211	-	-	1.163	NS
		Distance[b]	0.3599	0.2414	1.491	-	-	1.433	NS
		T*D	-0.1681	0.3833	-0.439	-	-	0.845	NS

AB/PT: Ash Basin (coal fly ash) and Pond T (paired references site)

AP/ST: Acid Pond (coal pile run-off) and Steel Creek Bay (paired reference site)

PA/TB: Pond A (radiocesium) and Thunder Bay (pair reference site)

[a] variable is treated as a binomial: 0m and >0m away from the water's edge

[b] variable is treated as continuous

Table 2.2 Indicator species analysis of carrion-associated beetle assemblages collected at the Savannah River Site in Aiken, South Carolina, USA during the summers of 2013-2014.

Site[a]	**Group(s)**	**Species**	**Indicator Value**	**P-Value**
AB/PT	AB	*Creophilus maximus*	0.791	0.002
AB	100 + 200 + 300m	*Deltochilum gibbosum*	0.975	0.002
	100 + 200 + 300m	*Nicrophorus orbicollis*	0.957	0.001
	100 + 200 + 300m	*Omorgus monachus*	0.893	0.006
	100 + 200 + 300m	*Onthophagus striatulus*	0.827	0.003
	100 + 200 + 300m	*Copris minutus*	0.781	0.016
PT	100 + 300m	*Trox foveicollis*	0.818	0.021
	100 +200 + 300m	*Deltochilum gibbosum*	0.955	0.001
	100 +200 + 300m	*Copris minutus*	0.909	0.001
AP/ST	AP	*Onthophagus hecate*	0.84	0.002
AP	100 + 200 + 300m	*Nicrophorus orbicollis*	0.987	0.002
ST	0 + 100m	*Onthophagus hecate*	0.825	0.036
	0 + 100 + 200m	*Hister coenosus*	0.875	0.026
	100 + 200 + 300m	*Nicrophorus orbicollis*	0.941	0.029
	100 + 200 + 300m	*Canthon chalites*	0.915	0.018
PA/TB	PA	*Onthophagus hecate*	0.821	0.006
PA	0 + 100m	*Onthophagus hecate*	0.99	0.003
TB	0m	*Onthophagus hecate*	0.808	0.006
	100 + 200 + 300m	*Deltochilum gibbosum*	0.905	0.028
	100 + 200 + 300m	*Nicrophorus tormentosus*	0.882	0.008

[a]AB/PT: Ash Basin (coal fly ash) and Pond T (paired reference site)

AP/ST: Acid Pond (coal pile run-off) and Steel Creek Bay (paired reference site)

PA/TB: Pond A (radiocesium) and Thunder Bay (pair reference site)

Figure Legend

Figure 2.1 Locations of sample collections on the Savannah River Site in South Carolina. Contaminated sites are the D-Area with Ash Basin and Acid Pond, and Pond A in R-Area. Uncontaminated sites are Steel Creek Bay, Pond T, and Thunder Bay.

Figure 2.2 Modified pitfall trap with rabbit carrion used as bait.

Figure 2.3 Individual-based rarefaction species accumulation graph of the site-pair Ash Basin/Pond T with traps pooled at the Savannah River Site in 2013.

Figure 2.4 Individual-rarefaction species accumulation graph of the site-pair Ash Basin/Pond T at the Savannah River Site in 2013.

Figure 2.5 Individual-rarefaction species accumulation graph of the site-pair Acid Pond/Steel Creek Bay with traps pooled at the Savannah River Site in 2014.

Figure 2.6 Individual-rarefaction species accumulation graph of the site-pair Acid Pond/Steel Creek Bay T at the Savannah River Site in 2014.

Figure 2.7 Individual-rarefaction species accumulation graph of the site-pair Pond A/Thunder Bay with traps pooled at the Savannah River Site in 2014.

Figure 2.8 Individual-rarefaction species accumulation graph of the site-pair Pond A/Thunder Bay at the Savannah River Site in 2014.

Figure 2.9 Dendrogram of similarity in species composition using hierarchal cluster analysis of the site-pair Ash Basin/Pond T at the Savannah River Site in 2013.

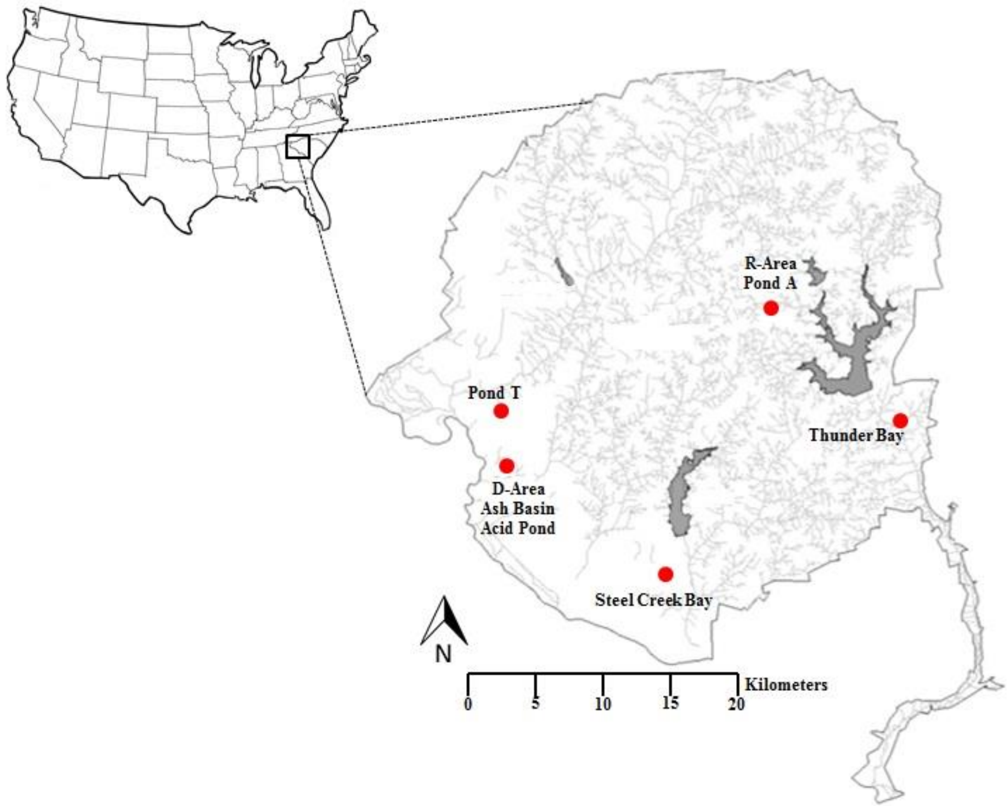

R-Area
Pond A
Pond T
Thunder Bay
D-Area
Ash Basin
Acid Pond
Steel Creek Bay
N
0
5
10
15
20
Kilometers

Figure 2.1

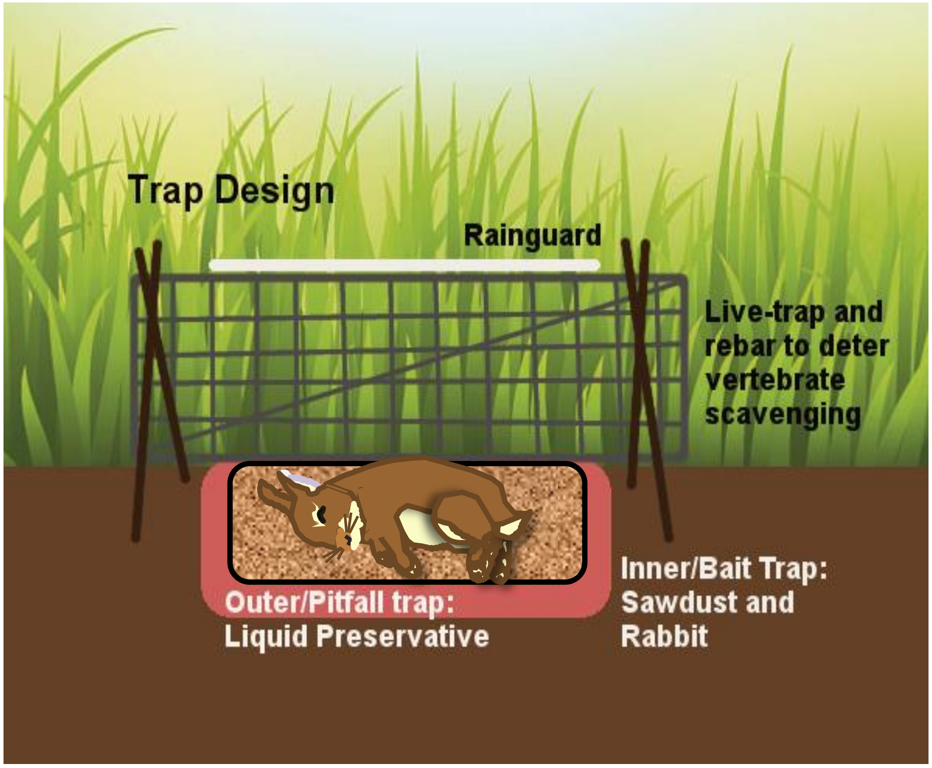

Trap Design
Rainguard
Live-trap and rebar to deter vertebrate scavenging
Inner/Bait Trap: Sawdust and Rabbit
Outer/Pitfall trap: Liquid Preservative

Figure 2.2

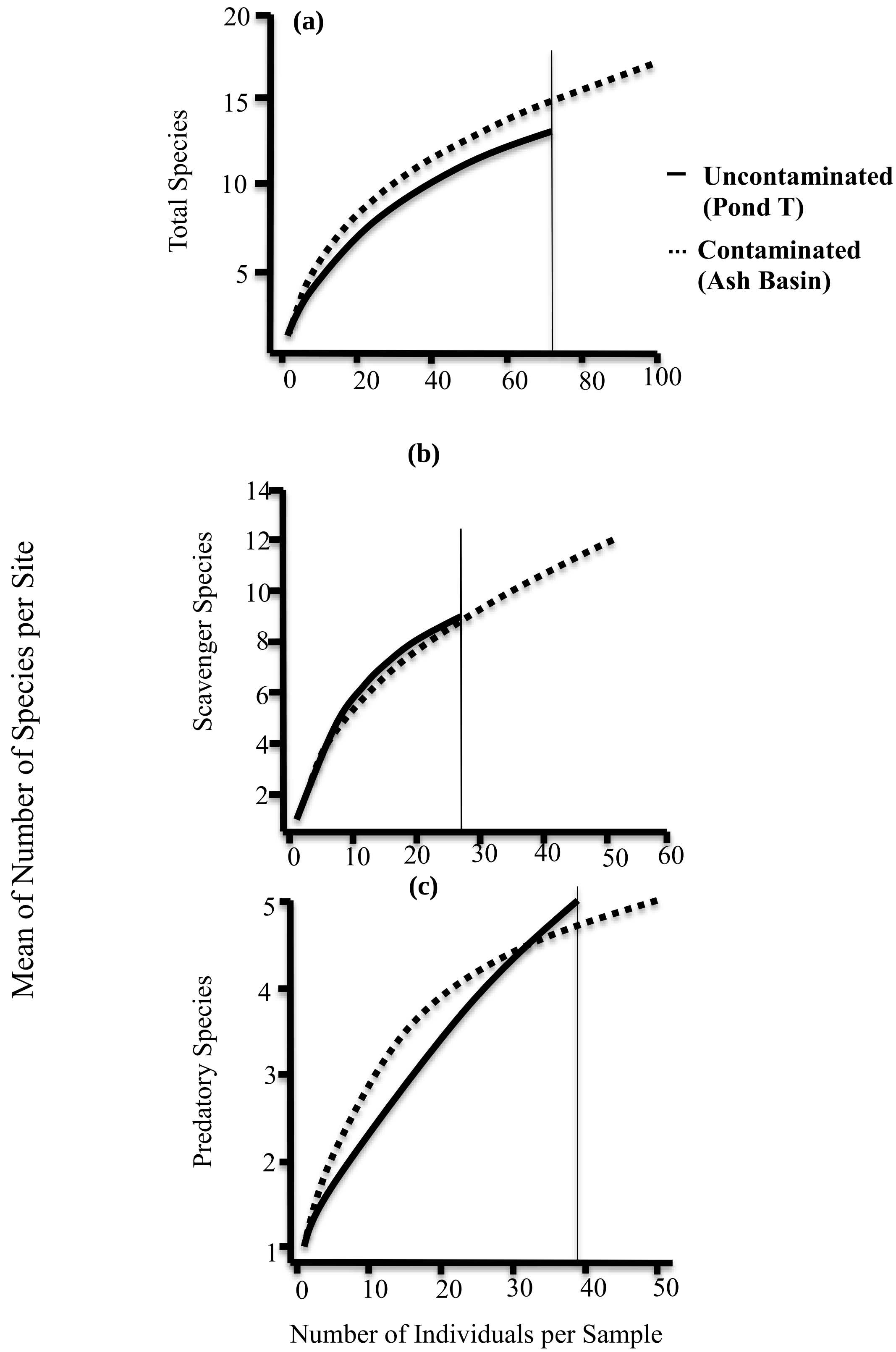

Mean of Number of Species per Site
(a)
Total Species
20
15
10
5
0
20
40
60
80
100
Uncontaminated
(Pond T)
Contaminated
(Ash Basin)
(b)
Scavenger Species
14
12
10
8
6
4
2
0
10
20
30
40
50
60
(c)
Predatory Species
5
4
3
2
1
0
10
20
30
40
50
Number of Individuals per Sample

Figure 2.3

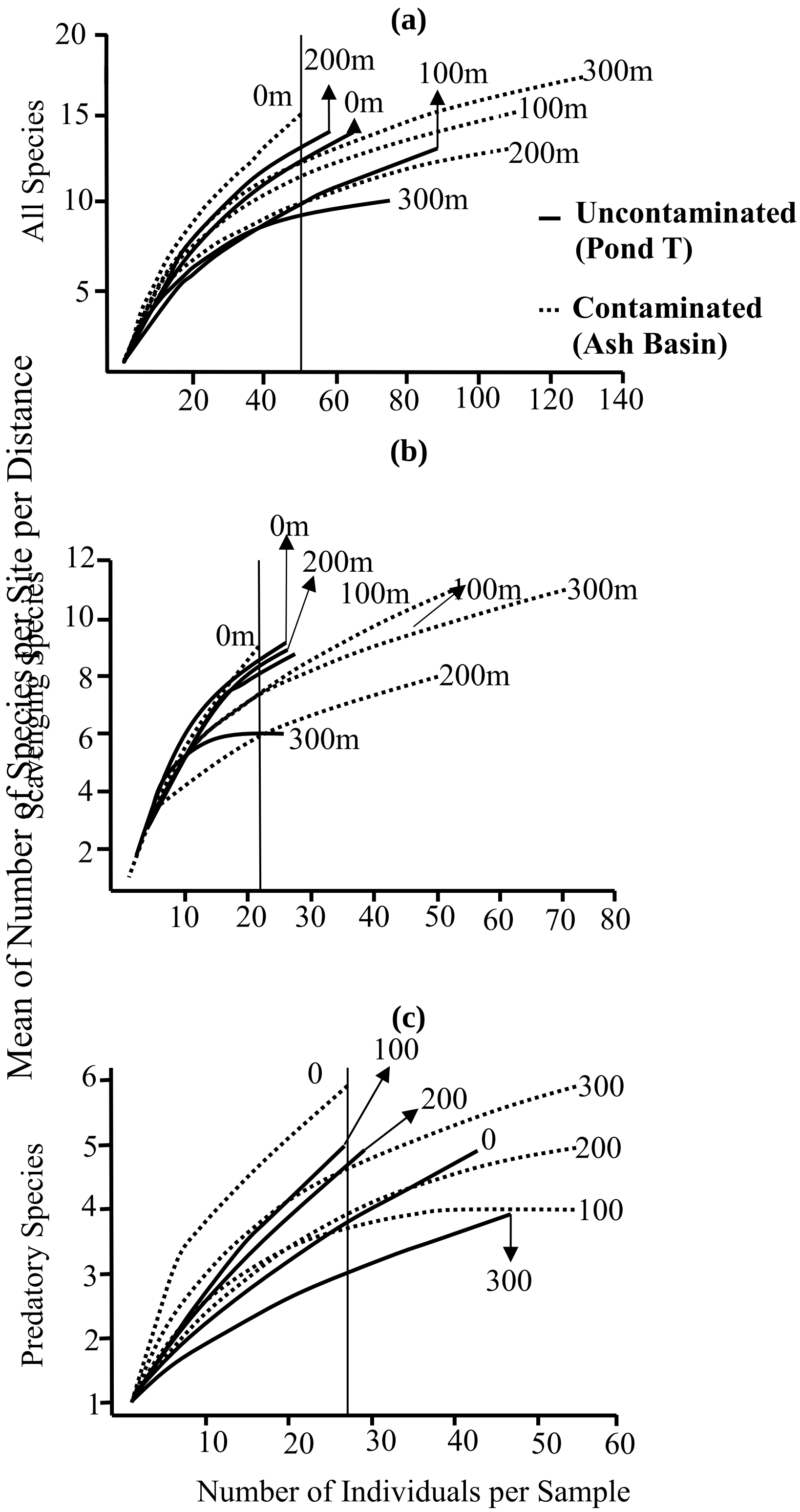

(a)
All Species
0m
200m
0m
100m
300m
100m
200m
300m
Uncontaminated (Pond T)
Contaminated (Ash Basin)
(b)
Mean of Number of Species per Site per Distance
Scavenging Species
0m
200m
100m
100m
300m
0m
200m
300m
(c)
Predatory Species
0
100
200
300
0
200
100
300
Number of Individuals per Sample

Figure 2.4

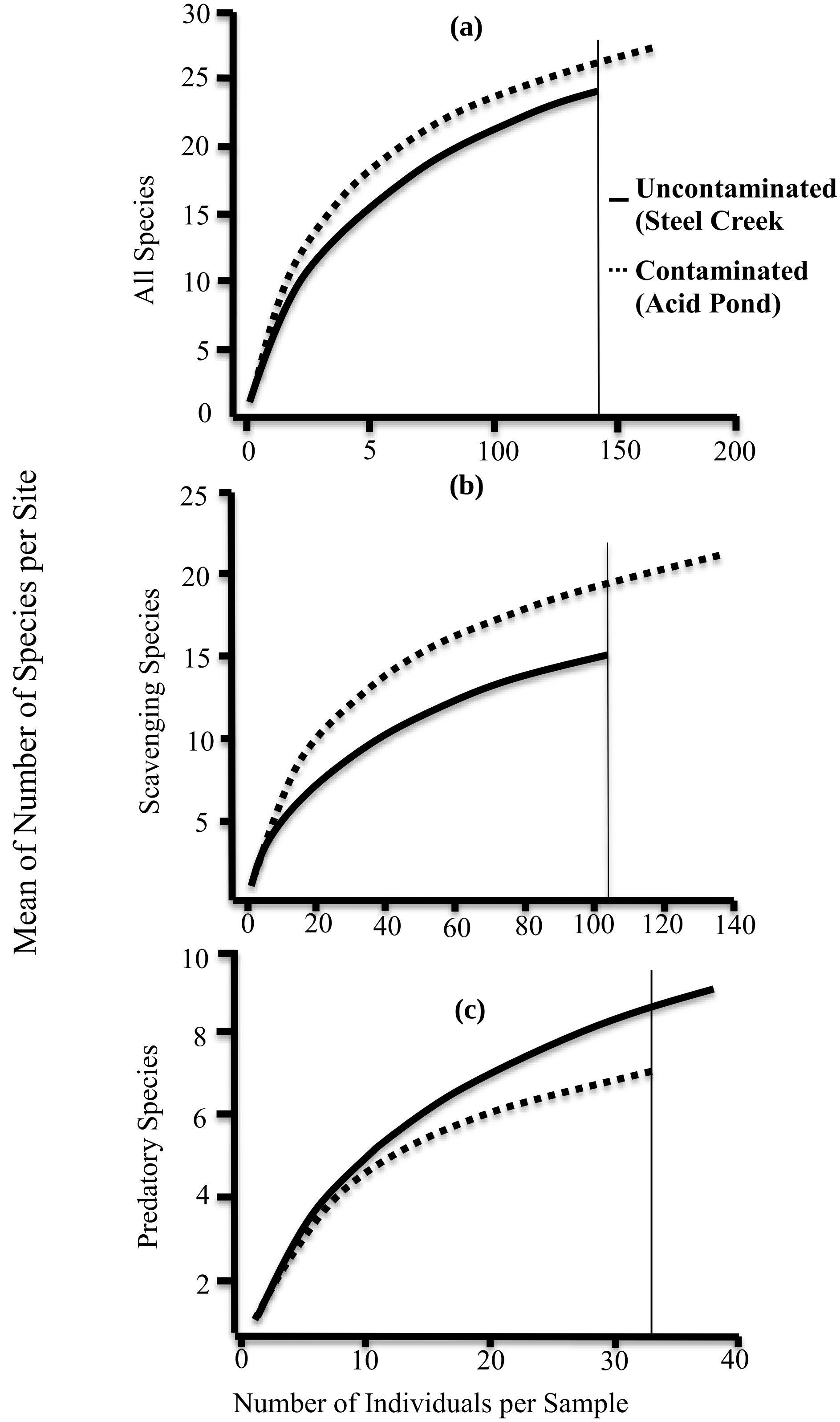

Mean of Number of Species per Site
(a)
All Species
0
5
10
15
20
25
30
0
5
100
150
200
Uncontaminated
(Steel Creek
Contaminated
(Acid Pond)
(b)
Scavenging Species
5
10
15
20
25
0
20
40
60
80
100
120
140
(c)
Predatory Species
2
4
6
8
10
0
10
20
30
40
Number of Individuals per Sample

Figure 2.5

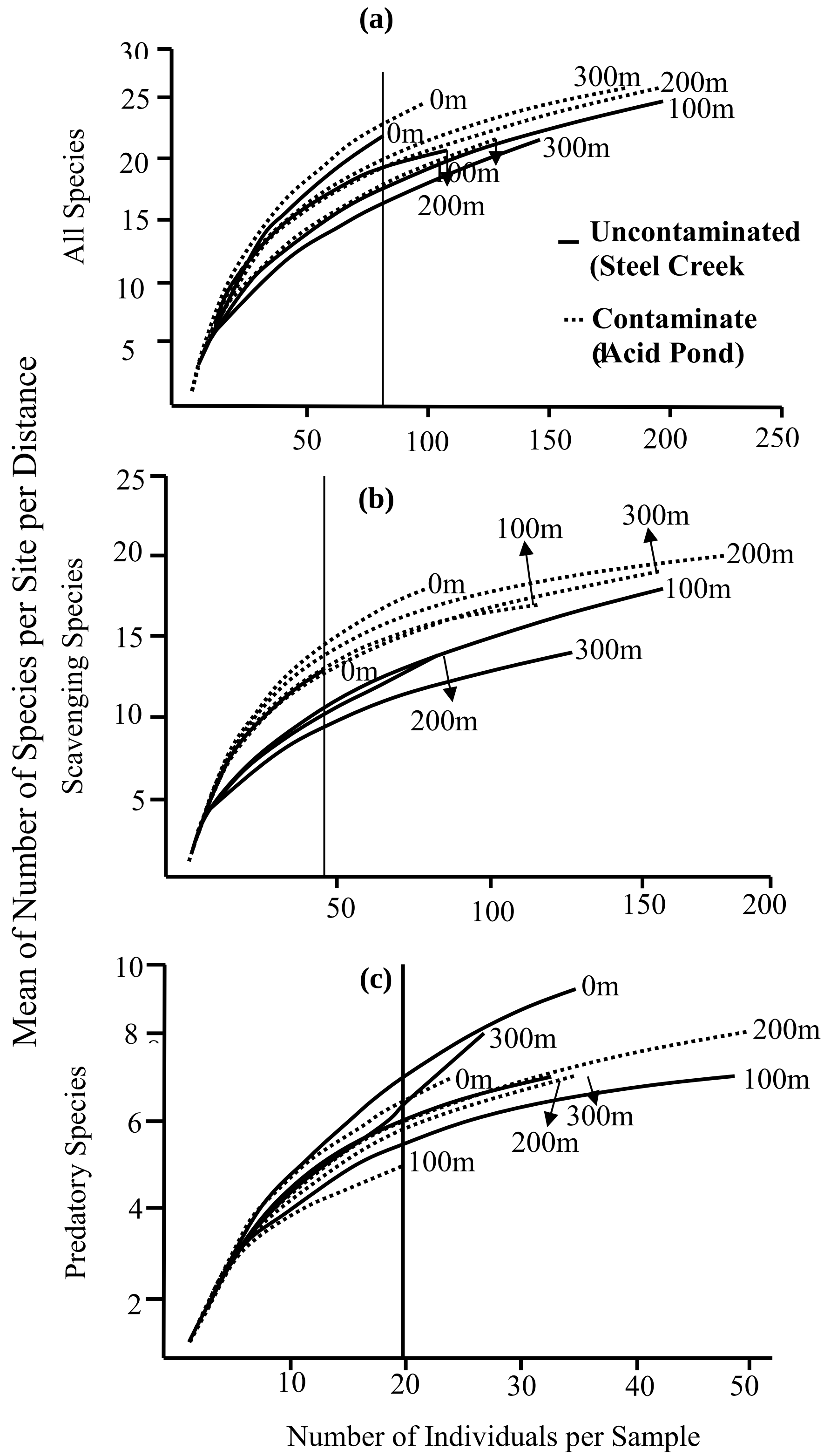

(a)
All Species
Uncontaminated
(Steel Creek
Contaminate
(Acid Pond)
0m
0m
300m
200m
100m
300m
100m
200m
(b)
Scavenging Species
100m
300m
200m
0m
100m
0m
300m
200m
(c)
Predatory Species
0m
200m
300m
0m
100m
300m
200m
100m
Mean of Number of Species per Site per Distance
Number of Individuals per Sample

Figure 2.6

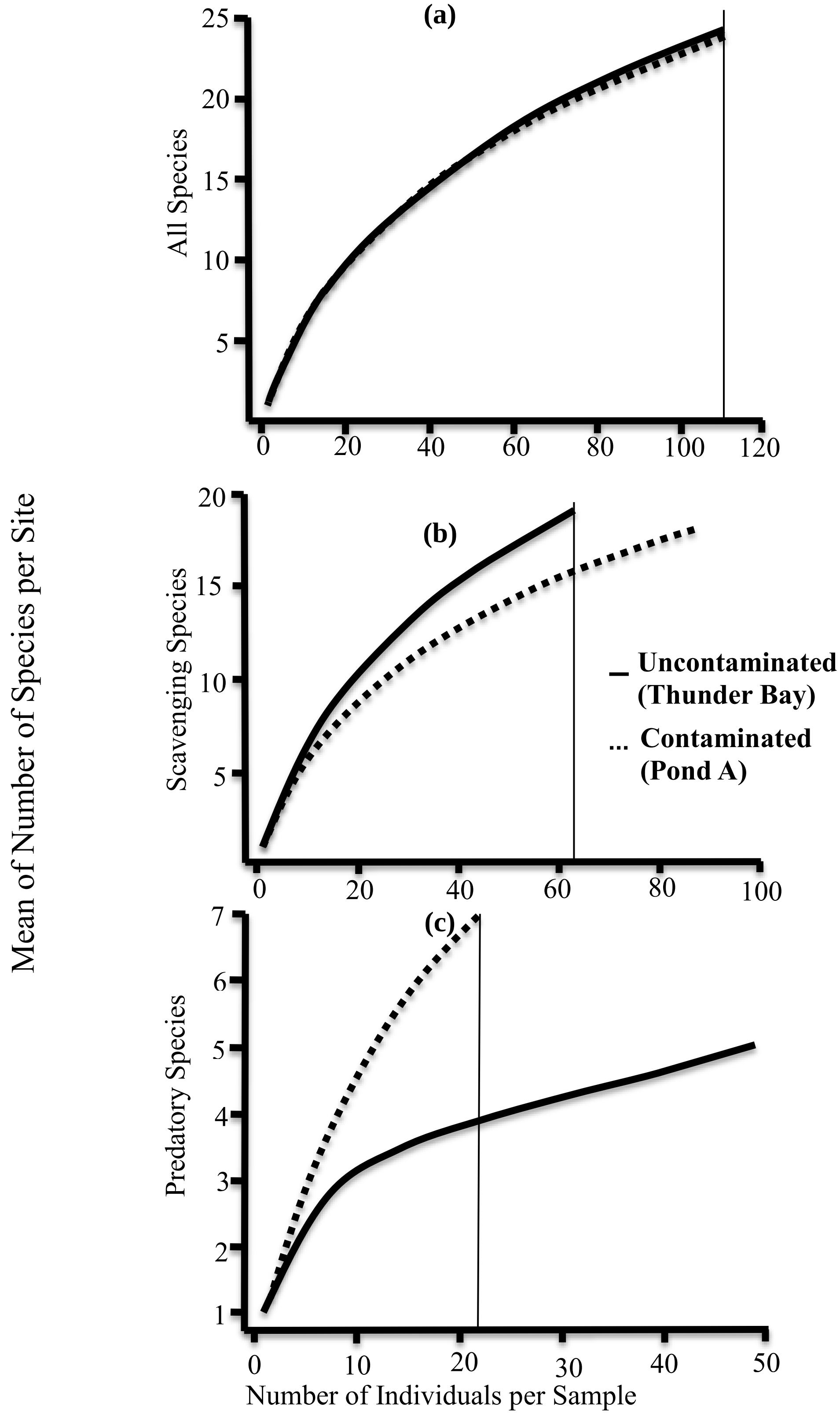

Mean of Number of Species per Site
(a)
All Species
25
20
15
10
5
0
20
40
60
80
100
120
(b)
Scavenging Species
20
15
10
5
0
20
40
60
80
100
Uncontaminated (Thunder Bay)
Contaminated (Pond A)
(c)
Predatory Species
7
6
5
4
3
2
1
0
10
20
30
40
50
Number of Individuals per Sample

Figure 2.7

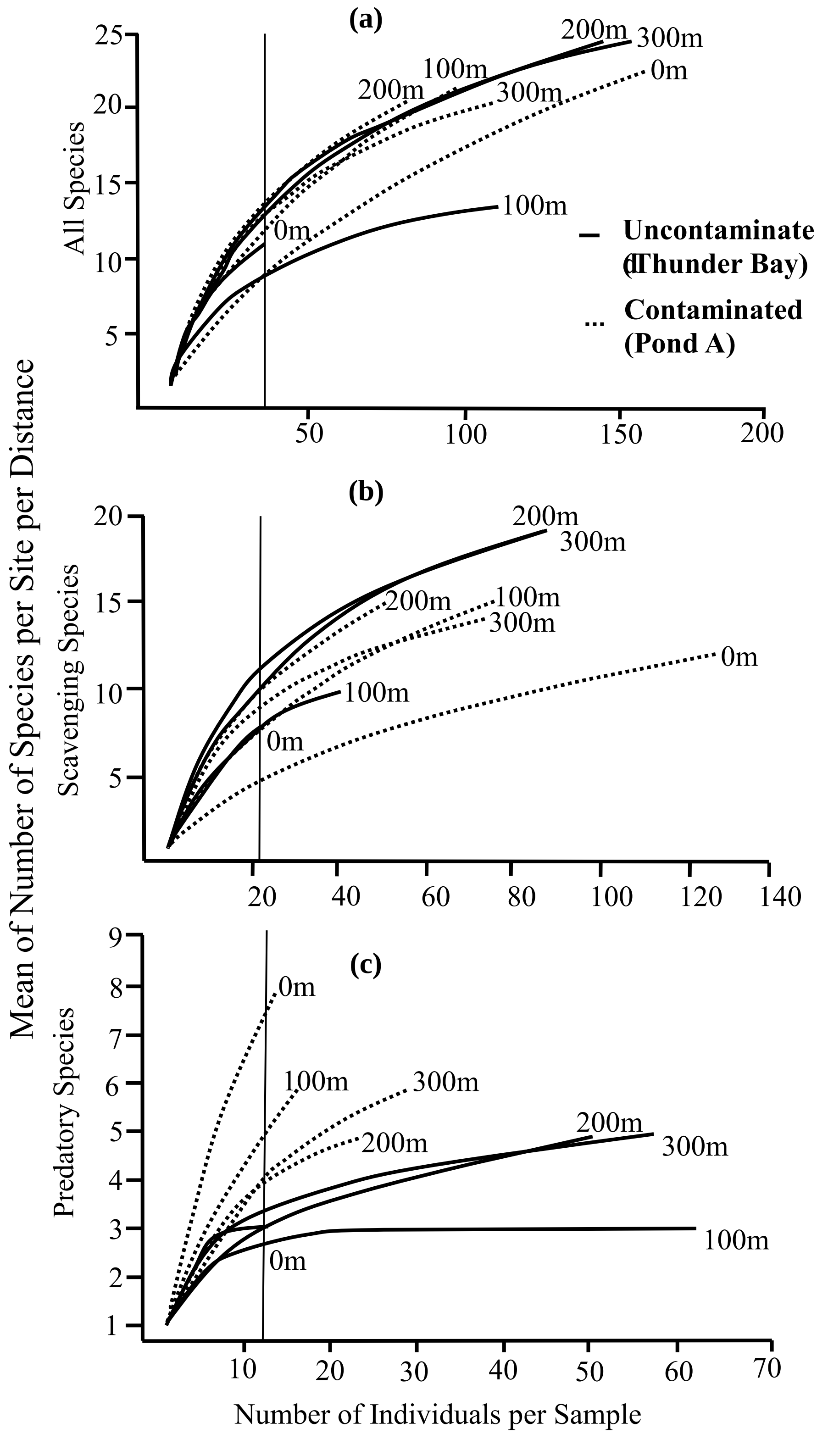
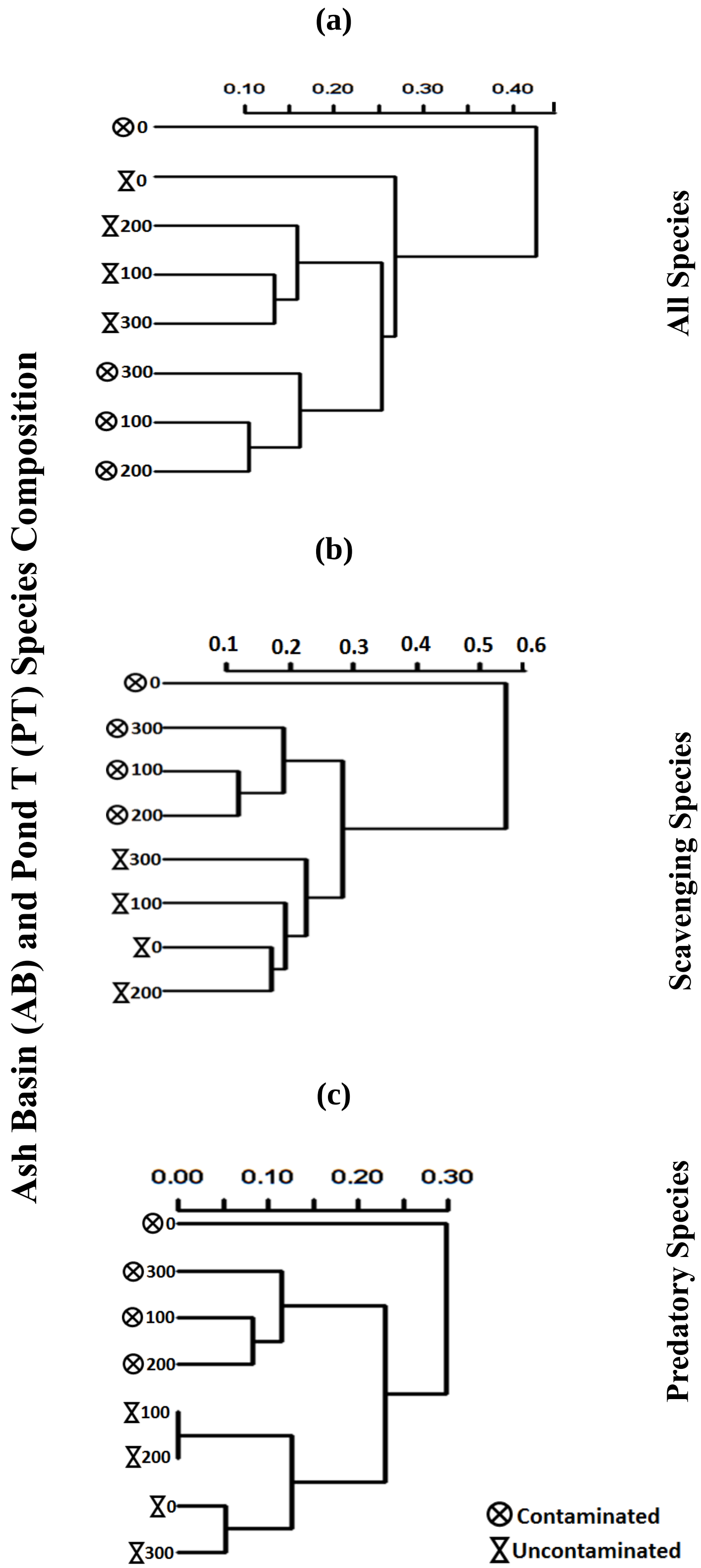

(a)
All Species
Mean of Number of Species per Site per Distance
Uncontaminate (Thunder Bay)
Contaminated (Pond A)
200m
300m
100m
0m
(b)
Scavenging Species
(c)
Predatory Species
Number of Individuals per Sample

Figure 2.8



Ash Basin (AB) and Pond T (PT) Species Composition
(a)
0.10
0.20
0.30
0.40
0
0
200
100
300
300
100
200
All Species
(b)
0.1
0.2
0.3
0.4
0.5
0.6
0
300
100
200
300
100
0
200
Scavenging Species
(c)
0.00
0.10
0.20
0.30
0
300
100
200
100
200
0
300
Predatory Species
Contaminated
Uncontaminated

Figure 2.9

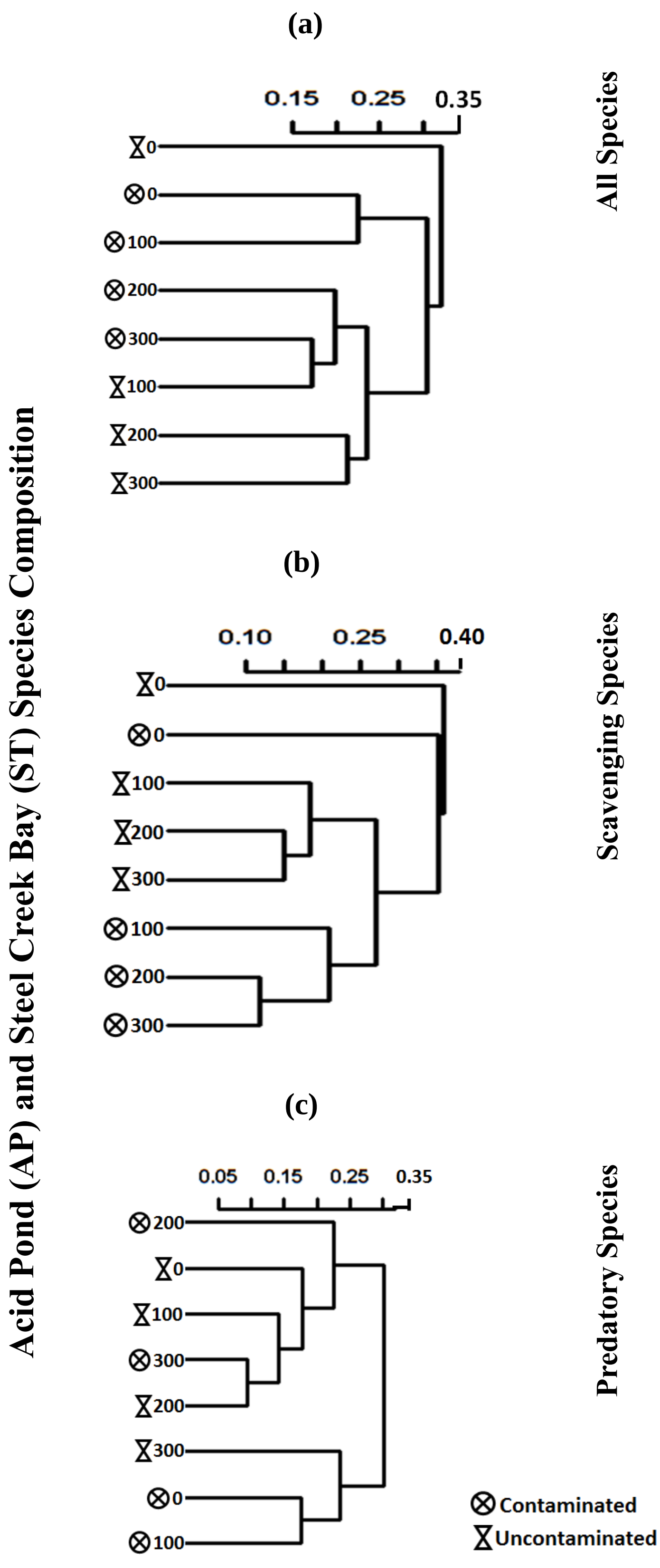

(a)
All Species
0.15
0.25
0.35
0
0
100
200
300
100
200
300
(b)
Scavenging Species
0.10
0.25
0.40
0
0
100
200
300
100
200
300
(c)
Predatory Species
0.05
0.15
0.25
0.35
200
0
100
300
200
300
0
100
Contaminated
Uncontaminated
Acid Pond (AP) and Steel Creek Bay (ST) Species Composition

Figure 2.10

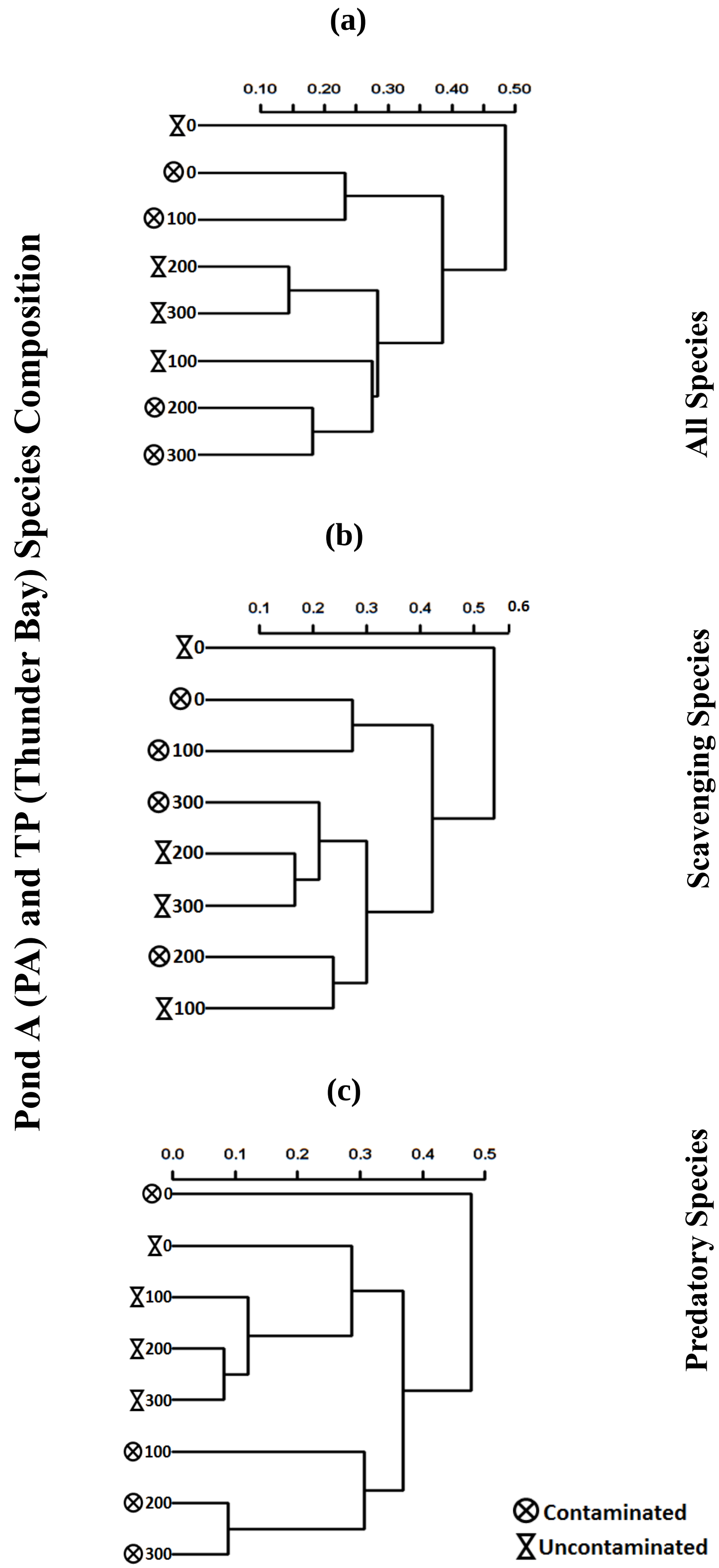

Pond A (PA) and TP (Thunder Bay) Species Composition
(a)
0.10 0.20 0.30 0.40 0.50
0
0
100
200
300
100
200
300
All Species
(b)
0.1 0.2 0.3 0.4 0.5 0.6
0
0
100
300
200
300
200
100
Scavenging Species
(c)
0.0 0.1 0.2 0.3 0.4 0.5
0
0
100
200
300
100
200
300
Predatory Species
Contaminated
Uncontaminated

Figure 2.11

CHAPTER 3

BIOACCUMULATION OF CONTAMINANTS OF SCARABAEIDAE AND SILPHIDAE BEETLES AT SITES POLLUTED BY COAL COMBUSTION RESIDUALS AND RADIOCESIUM

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Abstract

Anthropogenic contamination from coal-fired power plants and nuclear reactors is a pervasive issue impacting ecosystems across the globe. As a result, studies assessing the accumulation and effects of trace elements and radionuclides in a diversity of biota remains an active area of research. In particular, bioindicator species are routinely utilized as powerful tools for risk assessment of chemically contaminated habitats. Using inductively coupled plasma mass spectrometry (ICP-MS) and auto-gamma counting, we analyzed trace element and radiocesium concentrations in Scarabaeidae and Silphidae beetles, important species in decomposition and nutrient cycling, at contaminated and reference sites on the Savannah River Site, South Carolina, USA. Our results revealed variability in trace element concentrations between Scarabaeidae and Silphidae beetles, and between uncontaminated and contaminated sites. Compared to Scarabaeidae, Silphidae had higher levels of Chromium (Cr), Copper (Cu), Iron (Fe), Manganese (Mn), Nickel (Ni), and Zinc (Zn). Further, concentrations of Cr, Cu, and Ni were higher in both taxa at the uncontaminated site. However, Arsenic (As) was consistently high in both groups of beetles and Selenium (Se) in Scarabaeidae at the coal combustion waste site. Radiocesium concentrations were elevated in two individuals from a contaminated site. Our results suggest carrion beetles may be particularly sensitive to bioaccumulation and biomagnification of contaminants due to their trophic position and role in decomposition, and thus are useful sentinels of trace element and radionuclide contamination.

INDEX WORDS bioaccumulation, inductively coupled plasma mass spectroscopy, radiocesium, Scarabaeidae, Silphidae, Savannah River Site, trace elements

3.1 Introduction

Chemical contamination is a pervasive environmental issue that can have adverse effects on organisms, ecosystems, and consequently, humans. Moreover, anthropogenic activities have transformed the way chemical elements are distributed on the planet by artificially concentrating them through burning fossil fuels, smelting metals from ore, and applying fertilizers and pesticides. Some of these elements have converted from being essential nutrients to highly toxic substances that are listed as priority pollutants by the U.S Environmental Protection Agency (US-EPA 2016b). For example, products resulting from coal combustion like coal fly ash have a range of concentrated essential and nonessential elements such Arsenic (As), Cadmium (Cd), Chromium (Cr), Copper (Cu), Selenium (Se), Strontium (Sr), and Zinc (Zn) (Rowe et al. 2002). It is estimated that the U.S. generates 110 million tons of coal combustion waste every year, and has 735 active impoundments for retaining this waste (US-EPA 2015). Wildlife are generally not excluded from these impoundments and organisms utilizing these habitats have been demonstrated to accumulate substantive amounts of trace elements within their organs and other tissues (Lemly and Skorupa 2012). Further, accidents such as the coal fly ash spill at the Tennessee Valley Authority Kingston coal-fired power plant into the Emory River in 2008 can result in long-term ecological impacts affecting entire ecosystems (Ruhl et al. 2012).

In addition to coal combustion residuals, human activities have increased the magnitude of ionizing radiation in the environment on a global scale through nuclear weapons testing and accidents (Chernobyl and Fukushima). For example, in 1986 the Chernobyl nuclear reactor disaster released large amounts of radioactive material, including radiocesium (^{137}Cs) throughout much of the northern hemisphere (Dreicer et al. 1996). Due to its long physical half-life, radiocesium has wide-ranging and long-term adverse effects that are difficult to predict (Hinton et al. 2007). The ecological impacts of elevated exposure to radionuclides due to anthropogenic activities have long been a bewildering area of research for scientists. Although the introduction of radionuclides into the environment is less common than trace elements, the short- and long-term effects can be expansive and have serious implications spanning from cellular to ecosystem levels (Woodwell 1962).

Regulatory framework for risk assessment and bioremediation can be based on methods that do not provide accurate predictions (Traina and Laperche 1999). This is because ecosystems are inherently complex and thus unifying concepts can be difficult to identify and bioaccumulation models typically must incorporate numerous assumptions (Luoma and Rainbow 2005). Characterizing the levels of contaminants in the environment includes measuring the extent to which ecological receptors are exposed to contamination (i.e., bioavailability). Bioavailability is governed by many factors including the diverse reactions of a contaminant depending on environmental attributes such as the complexity of the local soil composition and pH (Van Gestel and Van Dis 1988, Lock and Janssen 2001). In addition, element-specific aspects such as uptake and elimination rates can contribute to consistent differences in bioavailability. For example, Silver (Ag) demonstrates a wide spectrum of bioaccumulation, ranging from very low concentrations in some organisms to very high in others (Luoma and Rainbow 2005). Due to the complexity of environmental influences, varying responses of different species and condition of a particular element, effects of specific elements are difficult to predict.

Adding to the complexity, elements rarely occur individually and their effects are often cumulative.

Another factor that governs bioavailability is the range of responses by different organisms, as biota bioaccumulate trace elements at different rates (Gutenmann et al. 1976, Patrick et al. 1976, Cherry and Guthrie 1977). While some species may demonstrate mechanisms for contaminant tolerance and detoxification strategies such as the formation of metal-binding proteins and rapid metal excretion, others may be unable to adapt and suffer reduced abundance or local extinction (Cain et al. 2004). Even in the absence of anthropogenic contamination, there can be high inter-taxa variability in trace element concentrations within the body and tissues (Rainbow 1988). Although many studies have focused on contaminant accumulation in vertebrates, soil arthropods also have been shown to bioaccumulate numerous contaminants (e.g., Janssen and Hogervorst 1993, Roth 1993, Rabitsch 1994, Van Straalen et al. 2001, Blanuša et al. 2002, Stone et al. 2002). Accumulation of contaminants within the bodies of invertebrates can be influenced by habitat-type and species, and may positively correlate with contaminant levels in the local environment (Mietelski et al. 2010, Ayabe et al. 2013).

It is well established that trace elements and radionuclides transfer through various trophic levels within food webs (e.g. Cherry and Guthrie 1977, Roberts and Johnson 1978, Butovsky and Van Straalen 1995, Jelaska et al. 2007). For example, among invertebrates, detrital feeders can accumulate higher levels of radiocesium when compared to other invertebrates in the same area and activity concentrations can vary seasonally (higher in winter and autumn)—reflecting a change in diet (Copplestone et al. 1999). Thus, beetles can be useful bioindicators of radioactivity and other contaminants as they have been shown to accumulate numerous contaminants and can be relatively easily sampled (Mietelski et al. 2003, Hodkinson and Jackson 2005).

Due to the vast range of responses to contaminants among different species, interspecific comparisons of levels of contaminants are generally not reliable (Rainbow 2002). However, to understand the pervasiveness of anthropogenic contamination and its effect on ecosystem functions, a suite of bioindicators may be taken into consideration. Little is known about the contaminant sensitivities of scavenging beetles (Coleoptera: Scarabaeidae; Silphidae) and their value as bioindicators of contamination from coal combustion waste and radiocesium. However, given their trophic position and role in the cycling of nutrients, scavenging beetles likely represent important sentinel species to elucidate of the presence of contaminants and potential impacts to ecosystem function.

3.2 Research Objective

Our research objective was to determine the extent to which Scarabaeidae and Silphidae beetles occupying contaminated habitats bioaccumulate trace elements and radiocesium. We hypothesize that individuals captured at contaminated sites would exhibit higher concentrations of those contaminants associated with the anthropogenic inputs (e.g., trace elements elevated in coal-fly-ash such as As, Se, and Tl). We focused both on areas contaminated with trace elements from coal combustion wastes and radiocesium from effluents of a nuclear reactor, and compared contaminant concentrations to those observed from a nearby uncontaminated reference area.

3.3 Methods

3.3.1 Study Area

We sampled beetles associated with carrion in west-central South Carolina at the Savannah River Site (SRS), which is one of the nuclear defense complex sites operated by the U.S. Department of Energy (DOE). The SRS is a limited-access area and research facility of approximately 800 km^2 designated during the 1950s for producing material for nuclear weapons. Although the nuclear reactors, heavy water extraction facility, and the nuclear fuel and target fabrication facility were all decommissioned by the late 1980s, these activities resulted in the release of numerous contaminants into several aquatic systems on the SRS (White and Gaines 2000). As a result, DOE and the U.S. Environmental Protection Agency (EPA) have identified 515 waste sites grouped into over 90 areas at the SRS, although much of the SRS landscape was not contaminated by DOE industrial activities (US-EPA 2016b). Many of the contaminated sites have been/are in the process of being remediated, however clean-up of various types of contaminants is currently on-going.

Aside from the industrial and administrative facilities, ~ 81% of the SRS is dominated by forested habitat. Located in the Sand Hills region within Aiken, Barnwell, and Allendale counties in South Carolina, dominant forest cover-types include loblolly (*P. taeda* L.) and longleaf (*P. palustris* Mill) pines as well as oak-hickory (*Quercus* and *Carya*) and tupelo-cypress (*Nyssa* and *Cupressus*) trees (White and Gaines 2000, DeVault et al. 2003). Aquatic habitats utilized by local fauna at SRS include bottomland swamps, streams, Carolina Bays, upland depressions, and two large cooling reserves (Lide 1994). Major soil series are Blanton, Fuquay, Lakeland, Orangeburg, and Vaucluse

(Serkiz 1998). In 2014, recorded temperatures averaged 28.23 °C with the maximum temperature reaching 42.78 °C.

3.3.2 Sampling Sites

We sampled beetles from four locations on the SRS: 1) Ash Basin (coal-fly ash – trace element contamination); 2) Acid Pond (coal run-off acid – trace element contamination); 3) Pond A (radiocesium contamination); and 4) Steel Creek Bay (uncontaminated control). Due to their close proximity (> 1 km) and similarity of coal-related contamination, samples from the Ash Basin and Acid Pond were pooled together. Although trace elements and radiocesium at the contaminated sites are concentrated in bodies of water, trophic interactions provide pathways for transferring contaminants from aquatic to terrestrial ecosystems and elevated contaminant burdens have been observed in numerous terrestrial species (Rowe et al. 2002, Ayabe et al. 2014).

The Ash Basin and Acid Pond are located at the D-Area of the SRS, which had a heavy water facility producing deuterium oxide and a coal-fired powerhouse. Although the coal-fire powerhouse has been retired and coal combustion waste is being consolidated and ash basins capped (US-EPA 2016a), the location still contains considerable trace element contamination within the basins and surrounding wetlands (Gaines et al. 2002). Depending on the ore body from which the coal was derived, coal by-products contain concentrated amounts of certain bioavailable traces metals such as Arsenic (As), Cadmium (Cd), Chromium (Cr), Copper (Cu), Selenium (Se), and Strontium (Sr) (Rowe et al. 2002). The coal related storage areas at D-Area include a coal storage run-off containment basin (Acid Pond) and a series of coal fly ash basins

(Ash Basin). The Ash Basin is a series of impoundments for retaining coal fly ash, a by-product of burning coal. In the 1950's, Beaver Dam Creek received thermal effluents from cooling water operations at D-Area which released a suite of metals into this nearby creek. Leachate from coal piles at D-Area has resulted in the low pH of an adjacent wetland (Carlson 1990), hence the name "Acid Pond". Previous studies have assessed bioaccumulation of trace elements in a diversity of biota at these sites (e.g., Brofft et al. 2002, Rowe et al. 2002).

Pond A is an impoundment located along a cooling water outflow canal created for R-reactor. It received effluent waters containing ^{137}Cs from the nearby nuclear reactor between 1954 and 1964 (Abraham et al. 2000). Aquatic ecosystems in the SRS have received over 2.09×10^{13} Bq of radiocesium, and as of 1996, it has been estimated that Pond A has a total ^{137}Cs inventory of 4.1×10^{10} Bq. The total area of Pond A is 5.2 ha with a depth of approximately 0.46 m (Carlton et al. 1992).

Steel Creek Bay is a relatively undisturbed isolated wetland depression in the southeastern quadrant of the SRS. It is one of hundreds of freshwater wetland habitats known as Carolina Bays that characterize the region. Similar to the contaminated sites, Steel Creek Bay is surrounded by various pines and mixed upland and bottomland hardwoods. Steel Creek Bay is approximately 0.48 ha in size (Schalles et al. 1989).

3.3.3 Beetle Collection

At each of our four sites, we established six trap locations that were >75 m apart, for a total of 24 traps. All traps were adjacent to the edge of the contaminated and uncontaminated water source (0 m). Beetles were collected using modified pitfall traps in June 2015. The trap consisted of a 5.7 L basin placed inside a larger 26.5 L basin. The trap was placed in the soil with the rim of the outer basin flush with the topsoil to also allow capture of beetles travelling along the ground. Drainage holes were made on all sides of the 26.5 L basin to avoid flooding during rain. We used medium-sized rabbit carcasses (0.49-2.48 Kg) as the carrion bait, obtained from Rodent Pro, a commercial breeder. Rabbits were euthanized via cervical dislocation and frozen by Rodent Pro. Carcasses were thawed indoors to ambient temperature and transported to the field in a sealed container to avoid invertebrate colonization prior to deployment in the field. We placed carrion within the 5.7 L basin on a substratum of 5 cm sawdust. A substratum of 3 cm of sawdust was also added to the outer basin to provide the trapped live beetles with protection until collection. To protect the carrion and beetles from vertebrate scavengers, an empty Tomahawk Livetrap (Tomahawk Live Trap Co., Tomahawk, Wisconsin) was set over the basins and secured by four rebar stakes. Once rabbit carrion bait was installed, collection took place on the third and fifth days from installation. After collection, samples were frozen until preparation. Once samples were thawed they were cleaned with deionized water and identified to lowest taxonomic level (Arnett et al. 2001, 2002, Pearson et al. 2006).

Representatives of the families Scarabaeidae (*Deltochilum gibbosum* Fabricius), and Silphidae (*Nicrophorus orbicollis* Say, *N. carolinus* L., *Necrophila americana* L., and *Necrodes surinamensis* F.) were used in this study. These species were utilized due to their abundance and relatively large body size compared to other captured beetles. *Deltochilum gibbosum* were used to represent the family Scarabaeidae, whereas all Silphidae were pooled per trap due to the lower number of individuals for various species. Following species identifications, all specimens were freeze-dried for two days prior to elemental and radiocesium analysis to ensure complete desiccation.

3.3.4 Trace Element Analysis

Desiccated beetle samples from D-Area and Steel Creek Bay were prepared for inductively coupled plasma mass spectrometry (ICP-MS) using a double acid extraction method at the Center for Applied Isotope Studies at the University of Georgia, Athens. Individual beetles were ground in a laboratory mill grinder and placed in metal-free tubes. Each individual beetle sample was weighed, as the target weight for ICP-MS was 150-200 mg. Each sample weighed a minimum of 150 mg and when > 200 mg, a portion of the sample was removed until target weight was reached. Three milliliters of concentrated nitric acid (70%) was added to the sample, and 6 mcL of gold was added to prevent any mercury present in the sample from adhering and bonding to tube plastic walls. Subsequently, 2 ml of peroxide were added and all samples were sonicated for a minimum of 30 min. All samples were brought to 30 ml using 2% nitric acid. Samples were weighted, centrifuged for at least 15 minutes at 2500 rpm and manually ran through the ICP-MS. A minimal detectable limit was acquired for each element and levels recorded at parts per billion.

ISP-MS was used to determine the levels of trace elements in the Scarabaeidae and Silphidae samples. Trace elements included Arsenic (As), Cadmium (Cd), Chromium (Cr), Cobalt (Co), Copper (Cu), Iron (Fe), Lead (Pb), Magnesium (Mn), Mercury (Hg), Nickel (Ni), Nobelium (No), Selenium (Se), Thallium (Tl), Uranium (U), and Zinc (Zn). Software used with ICP-MS was ThermoElectron-PlasmaLab Version 2.5

from EISC. The ICP-MS mode selected was solution in conventional mode with corrections. A blank for quality control was run every 20 samples.

3.3.5 Radiocesium Analyses

Individual beetles of the family Scarabaeidae (*D. gibbsosum*) originating from Pond A (radiocesium) and Steel Creek Bay (reference) were freeze-dried and packed into 5 ml scintillation tubes and assayed on a Packard Cobra II auto-gamma counter (Model Cobra II 5003, Packard Instruments Co., Meriden, CT, USA). The instrument was calibrated using a NIST traceable stock solution. We randomly analyzed an empty tube as background (a blank) and each tube was counted for 60 minutes. Radiocesium levels were reported in Bq/g dry weight and the conversion factor was 27.027 for pCi to Bq. Minimum Detectable Activity (MDA) was calculated for each sample. A total of 25 individuals of *D. gibbosum* were analyzed from Pond A and 25 from the control site, Steel Creek Bay.

3.3.6 Data Analyses

Before analyses, all values were converted from parts per billion to parts per million (ppm). For each element, any value that was below the minimal detectable limit (MDL) for that element was changed to half of that element's mdl. Only elements for which > 50% of samples were above the mdl were included in subsequent analyses (Hall et al. 2009, Fletcher et al. 2014) Data were tested for normality using Shapiro-Wilk normality test in R statistical software, version 3.3.1 (R core team 2012). The data failed to meet normality assumptions after log and square root transformations. Thus, we used non-parametric Mann Whitney U Tests to elucidate differences in trace element concentrations between the two taxa (Scarabaeidae and Silphidae) from the uncontaminated site, and between the two sites (uncontaminated and D-Area) with pooled species and separately for the two species groups. Due to only two samples resulting in levels above the MDA for radiocesium (see results), statistical tests were not performed on these samples.

3.4 Results

From the samples collected at D-Area and Steel Creek Bay, a total of 73 individuals of Scarabaeidae (*D. gibbosum*) and 51 individuals of Silphidae (*N. americana*, *N. orbicollis*, *N. surinamensis*, and *N. carolinus*) were collected in traps. Of the 15 elements tested, Cadmium (Cd), Cobalt (Co), Nobelium (No), and Uranium (U) were excluded from statistical analyses since 50% of the values were below MDLs. Elements above the MDLs for more than 50% of samples included Arsenic (As), Chromium (Cr), Copper (Cu), Iron (Fe), Lead (Pb), Magnesium (Mn), Mercury (Hg), Nickel (Ni), Selenium (Se), Thallium (Tl), and Zinc (Zn).

Body burdens of elements varied between Scarabaeidae and Silphidae regardless of treatment-type (Table 3.1). Beetles from the family Silphidae had higher body burdens of Cr (585%), Mn (22%), Fe (335%), Ni (755%), Cu (40%), and Zn (26%) than Scarabaeidae. Beetles from the family Scarabaeidae were 27% and 74% higher in As and Se, respectively.

Mann Whitney U Tests determined that levels of six elements in all the beetle species pooled were different between the contaminated and uncontaminated sites (Table

3.2). Specifically, uncontaminated site had higher Cr (79%), Cu (18%), and Ni (124%) levels, whereas sites contaminated with trace elements from coal-fly-ash had higher As (203%), Se (110%), and Tl (17%) levels in beetles. Both Scarabaeidae and Silphidae had higher levels of As (72% and 1,319% higher, respectively) at the contaminated site (Table 3.3). At the contaminated site, Scarabaeidae had higher Se (175%), Tl (20%), and Fe (30%) compared to the uncontaminated site. At the uncontaminated site, Silphidae were 50% higher in Cu compared to the contaminated site.

For radiocesium, 25 individuals of *D. gibbosum* were analyzed each from Pond A and the control site, Steel Creek Bay. Although most samples were below MDA, two beetles from Pond A had elevated levels of radiocesium (1.16 Bq/g and 1.4 Bq/g).

3.5 Discussion

We investigated trace element and radiocesium concentrations in two families (Scarabaeidae and Silphidae) of carrion-associated beetles collected at contaminated and uncontaminated sites at the ~800 km^2 SRS in the coastal plain of west-central South Carolina. We found varying concentrations of trace elements between Scarabaeidae and Silphidae beetles. For example, Silphidae beetles were higher in Cr, Cu, Fe, Mn, Ni, and Zn. Possible reasons include variation in diets as even though *D. gibbosum* and Silphidae beetles are both scavengers, their dietary selection is distinct (Jelaska et al. 2007). *Deltochilum gibbosum* generally consumes excrement, feathers, and animal fur (Howden and Ritcher 1952) whereas beetles of the family Silphidae feed directly on the carcass flesh (Milne and Milne 1976). Contaminant concentrations of a carrion food source may differ between tissues, and feathers or fur, which could explain variability of contaminant concentrations in scavenging beetles with different dietary selection. Further, bioaccumulation may vary depending upon the element (e.g., some species are able to regulate Cu efficiently regardless of soil-levels), and it's possible that these trends reflect absorption/excretion levels of elements within different taxa (Stone et al. 2002).

Invertebrates have various detoxification methods to mitigate the effects of toxic elements (Hopkins 1989) including reduced body sizes (Jones and Hopkins 1998), lower reproduction (Lagisz et al. 2002), and increased respiration rates (Bednarska and Stachowicz 2013). Further, contamination can deplete an organism's energy reserves even after detoxification due to the necessity of repairing damage at the cellular level (Calow 1991). Depleted energy reserves can result in an organism's decreased tolerance to additional stressors (Sibly and Cowell 1989, Stone et al. 2001). The variability surrounding detoxification methods and its effects could provide some explanation for the variability in contaminant levels between Scarabaeidae and Silphidae beetles.

Substantive variability in element concentrations was also found between treatment-types. We found higher levels of As and Se in *D. gibbosum* and Silphidae beetles captured at the Ash Basin site, as has been reported in other taxa collected in habitats contaminated with coal fly ash (Guthrie and Cherry 1976, Cherry and Guthrie 1977, Lemly 2002, Roe et al. 2005, Snodgrass et al. 2004, Hopkins et al. 2006, Roe et al. 2006, Reash 2012, Otter et al. 2012, Van Dyke et al. 2013, Fletcher et al. 2014a, Fletcher et al. 2014b). In 1974, when coal ash was sluiced into the drainage basins at D-Area, various invertebrates, including Odonata and Coleoptera were found to have 2.1 and 2.6 ppm of As and Se, respectively (Cherry and Guthrie 1977). Our data suggest this site continues to be a source of elevated exposure to these elements as Siliphidae beetles at the Ash Basin had mean As concentrations of 4.882 ppm, whereas those from the uncontaminated site had mean concentrations of 0.344 ppm. We found similar trends for As in Scarabaeidae beetles as there were mean concentrations of 3.114 and 1.817 ppm at the contaminated and uncontaminated site, respectively. In our study, mean Se concentrations were higher at the contaminated (2.523 ppm) compared to the uncontaminated site (1.204 ppm), which has been found in other studies from the same contaminated site (Cherry and Guthrie 1997, Roe et al. 2006, Fletcher et al. 2014a). However, compared to Se concentrations in other invertebrates (Fletcher et al. 2014a) mean Se concentrations in our study may not represent substantial amounts in neither Scarabaeidae nor Silphidae beetles. The US-EPA has suggested a threshold of Se at 7.91 ppm below which most aquatic life should not be negatively impacted (US-EPA 2004). Although mean Se concentrations may not be substantial, the maximum range was ~17 ppm at the contaminated site, which likely represents toxic levels for beetles. Even though the levels of As concentrations between the two families were different, and mean Se concentrations may not be substantive, we conclude that both families are important sentinel species in elucidating the presence of As and Se and potential effects to ecosystem function,

Thallium (Tl) was found to be higher at the contaminated site and higher in Scarabaeidae than Silphidae beetles. Although Tl is rare in nature, it occurs naturally in Earth's crust (Keith and Telliard 1979). Yet, there are few studies on its bioaccumulation and ecological effects, even though it can be highly toxic (Heim et al. 2002, Dumas and Hare 2008). One reason for the limited research on Tl in the environment is its high analytical detection limit, which makes difficult to quantify accurately (Cheam 2001).

Our study found higher levels of Tl at the ash basin, which we hypothesized since coal combustion is a known source of elevated Tl concentrations (Smith and Carlson 1977). However, observed concentrations of Tl were below those considered to be toxic and thus the biological relevance of these levels in Scarabaeidae and Silphidae beetles is unclear (Borgmann et al. 1998).

We found that beetles trapped in uncontaminated sites had higher levels of Cr, Cu, and Ni (elements not associated with coal-fly-ash at our study site; Whittaker) than beetles trapped at sites contaminated with coal-fly-ash, suggesting that Steel Creek Bay may have a unique geochemical composition. Certain areas have naturally occurring elevated levels of elements like Cr, Cu, and Ni (Whittaker 1954) and elements such as these are natural constituents of the earth's crust. Weathering of parent rock results in heavy metals and metalloids, however these generally occur in trace amounts that rarely reach toxic levels (Kabata-Pendias 2001). Due to the long history of modern anthropogenic influences prior to the creation of SRS, especially agriculture, it is possible elevated levels of these elements observed in our reference site may reflect past land use history. Aside from naturally occurring elements and pollution from coal fly ash, toxic elements can accumulate in soils through other sources such as sewage sludge, pesticides, fertilizers, animal manure, and atmospheric deposition (Khan et al. 2008, Zhang, et al. 2010). Phosphate fertilizers, of which large quantities are added to the soil, are known to have trace amounts of heavy metals such as Cd, Hg, and Pd (Jones and Jarvis 1981). Other elements, like Cu are routinely added to soils through agricultural practices (Lasat 2000). Moreover, due to the anthropogenic influence on the planet's geochemical cycle, most of today's environments may be accumulating one or more elements above nontoxic background values (D'Amore et al. 2005).

Although radiocesium levels were below detection limits in most beetles sampled in this study, elevated levels were observed in two individuals of *D. gibbosum* collected from a site with known radionuclide contamination. A range of biota analyzed from this site has previously been shown to bioaccumulate high levels of radiocesium (Whicker et al. 1990). Moreover, Scarabaeidae beetles have been shown to bioaccumulate radionuclides in their inner organs; however patterns of radionuclide distribution among individuals are variable (Mietelski et al. 2003). In addition, variability in habitat-types can further contribute to variation in radionuclide levels (Ayabe et al. 2014). Areas with complex food webs such as those that connect aquatic and terrestrial habitats can have more variation in the levels of radionuclide bioaccumulation, particularly when contaminated inputs are primarily constrained to one habitat type, in this case the aquatic environment. Although our traps were placed adjacent to the contaminated water body, beetle exposure to radiocesium in this study is likely variable as vertebrate carrion can vary widely in contaminated levels depending on their trophic position and fidelity to the aquatic system. Additionally, although organisms can bioaccumulate radiocesium to elevated concentrations, it is generally removed from the body quickly, in some cases a matter of days or weeks (Rowan and Rasmussen 1995), which undoubtedly contributes to variability in body burdens of radiocesium observed in this study. Thus, it is likely that the two individuals of *D. gibbosum* with elevated radiocesium concentrations had recently consumed contaminated carrion. Future research is needed to determine the uptake and elimination rates of radiocesium in Scarabaeidae beetles based on a food resource with known radiocesium concentrations.

Given their unique trophic position and ability to accumulate elevated levels of multiple trace elements and radionuclides, our data suggests Silphidae and Scarabaeidae beetles represent important sentinel species to elucidate the presence of contaminants and potential impacts to ecosystem function. However, results from our study revealed variability in trace element concentrations amongst elements, treatments, and beetle taxa. Most ecotoxicology studies have focused on short-term effects of stressors (Van Staalen et al. 2007), which differ from observations of chronic exposure (Morgan et al. 2007). There are high energetic costs associated with living in chronically polluted environments and adaptations to mitigate such stress are common among invertebrates (Morgan et al. 2007). Regardless of the elevated levels of contaminants at our study sites, we observed robust reproductive populations of Scarabaeidae and Silphidae beetles. Due to the long history of contamination at the SRS, it is possible that naturally selected, metal and/or radiocesium-resistance ecotypes exists. Although levels of contamination observed within the beetles sampled in our study may not be at lethal levels, there may be biological costs (e.g., survivorship and reproduction) for managing effects of contaminant exposure and allocating resources for coping with these burdens (Calow 1991, Forbes and Calow 1996). Further studies may determine biomarkers, such as respiration rates in Scarabaeidae and Silphidae beetles for examining coping mechanisms for contaminant exposure. Additionally, investigations are needed to determine if Scarabaeidae and Silphidae have acquired genetically determined adaptive traits due to stress from trace elements and radiocesium contamination.

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Table 3.1 Comparison of trace element concentrations (ppm) for Scarabaeidae and Silphidae beetles collected in 2015 from Steel Creek Bay (uncontaminated site) at Savannah River Site, South Carolina.

	Scarabaeidae (N = 40)			**Silphidae (N =31)**				
Element[a]	**Mean**	**SE**	**Range**	**Mean**	**SE**	**Range**	***W***	***P***
Chromium	15.472	1.986	2.232-80.105	115.686	19.256	26.253-553.696	88	**<0.001**
Manganese	31.465	3.520	7.654-123.906	40.259	3.895	20.267-119.957	2292	**0.049**
Iron	200.430	14.345	23.163-622.992	1015.153	121.741	325.746-3042.599	108	**<0.001**
Nickel	6.872	1.229	0.528-43.105	57.385	9.206	9.354-242.555	133	**<0.001**
Copper	19.198	1.131	4.175-45.695	33.435	2.245	17.257-76.647	1683	**<0.001**
Zinc	99.240	17.254	19.350-752.568	124.271	4.958	70.451-197.378	1288	**<0.001**
Arsenic	1.875	0.298	0.129-8.660	0.322	0.063	0.095-2.058	5322	**<0.001**
Selenium	1.257	0.131	0.069-4.000	1.136	0.146	0.047-4.955	4182	**<0.001**
Mercury	0.439	0.154	0.004-6.343	0.626	0.203	0.032-5.474	2575	0.849
Thallium	0.004	0.0008	0.0002-0.031	0.009	0.002	0.0005-0.049	2537	0.096
Lead	0.712	0.513	0.029-21.408	0.165	0.014	0.585-0.510	2826	0.592

[a] Elements Cd, Co, No, and U were not included in this table since over 50% of their values were below minimum detection limits.

Table 3.2. Comparison of trace element concentrations (ppm) for total beetles collected from D-Area and Steel Creek Bay at the South Carolina in 2015.

	Steel Creek Bay (N= 71)			**D-Area (N= 93)**				
Element[a]	**Mean**	**SE**	**Range**	**Mean**	**SE**	**Range**	***W***	***P***
Chromium	59.230	10.303	2.232-553.696	33.030	5.753	2.351-410.468	2621	**0.024**
Manganese	35.261	2.433	7.654-123.906	33.108	1.369	12.853-83.667	3248	0.864
Iron	556.154	71.900	23.163-3042.546	395.395	51.168	75.299-3530.599	2902	0.185
Nickel	28.927	5.030	0.537-242.256	12.905	2.658	0.528-220.719	2334	**<0.001**
Copper	25.409	1.483	4.174.76.646	21.580	0.861	6.089-54.416	2617	**0.023**
Zinc	110.172	10.012	19.350-752.568	97.116	3.084	22.813-197.212	2930	0.218
Arsenic	1.165	0.189	0.094-8.660	3.530	0.979	0.073-86.628	4845	**<0.001**
Selenium	1.204	0.097	0.068-4.932	2.523	0.291	0.014-17.141	4782	**<0.001**
Mercury	0.521	0.124	0.032-6.343	0.284	0.030	0.004-1.756	2742	0.064
Thallium	0.006	0.001	0.0001-0.048	0.007	0.001	0-0.044	4297	**<0.001**
Lead	0.473	0.299	0.028-21.408	0.211	0.024	0.039-1.906	3488	0.537

[a] Elements Cd, Co, No, and U were not included in this table since over 50% of their values were below minimum detection limits.

Table 3.3 Comparison of trace element concentrations (ppm) between D-Area (contaminated) and Steel Creek Bay (uncontaminated) for beetles of the families Scarabaeidae and Silphidae collected from the Savannah River Site, South Carolina in 2015.

	Scarabs				**Silphids**			
	D-Area (n=73)	**Control (n=40)**			**D-Area (n=20)**	**Control (n=31)**		
Element[a]	**Mean (SE)**	**Mean (SE)**	***W***	***P***	**Mean (SE)**	**Mean (SE)**	***W***	***P***
Chromium	17.875 (2.24)	15.475 (1.98)	1618	0.414	95.697 (21.64)	115.687 (19.29)	240	0.182
Manganese	32.176 (1.37)	31.464 (3.05)	1612	0.440	37.219 (3.87)	40.161 (3.82)	291	0.724
Iron	260.444 (21.90)	200.431 (14.39)	1856	**0.026**	995.562 (189.65)	1015.153 (121.71)	257	0.314
Nickel	6.004 (0.75)	6.872 (1.23)	1383	0.567	40.265 (10.29)	57.385 (2.25)	214	0.065
Copper	21.358 (0.917)	19.191 (1.31)	1668	0.266	21.930 (2.23)	33.432 (2.25)	104	**<0.001**
Zinc	92.669 (3.212)	99.246 (17.25)	1679	0.239	112.689 (7.20)	124.27 (4.98)	239	0.175
Arsenic	3.119 (0.454)	1.817 (0.293)	2016	**<0.001**	4.882 (4.31)	0.344 (0.06)	445	**0.009**
Selenium	2.765 (0.33)	1.257 (0.132)	2248	**<0.001**	1.500 (0.55)	1.137 (0.15)	269	0.796
Mercury	0.266 (0.03)	0.439 (0.154)	1181	0.077	0.343 (0.08)	0.625 (0.023)	286	0.653
Thallium	0.006 (0.0006)	0.005(0.0009)	2048	**<0.001**	0.009 (0.002)	0.009 (0.002)	373	0.230
Lead	0.214 (0.03)	0.712 (0.531)	1619	0.411	0.213 (0.05)	0.165 (0.01)	314	0.947

[a] Elements Cd, Co, No, and U were not included in this table since over 50% of their values were below minimum detection limits.

CHAPTER 4

THESIS CONCLUSIONS

4.1 Conclusions

Carrion-associated beetles are valuable contributors to energy flow and nutrient recycling in terrestrial ecosystems. Previous studies on the effects of anthropogenic disturbances on carrion-associated beetles have focused mainly on deforestation and habitat fragmentation (Klein 1989, Lomolino et al. 1995, Lomolino and Creighton 1996, Trumbo and Bloch 2000, Gibbs and Stanton 2001, Wolf and Gibbs 2004) and little is known about the effects of anthropogenic contamination on carrion-associated beetles (Ermakov 2012). In this study, we collected scavenger and carrion-associated predatory beetles from contaminated and uncontaminated habitats on the U.S. Department of Energy's Savannah River Site in South Carolina to investigate levels of bioaccumulation and the effects of anthropogenic contaminants on the abundance and community composition of this important group of taxa. Inputs from industrial activities at the contaminated locations included coal combustion waste and radiocesium. Although these contaminants were primarily concentrated in aquatic systems at our study sites, numerous studies have demonstrated the bioavailability of these contaminants into various terrestrial taxa (Burger et al. 2002). In Chapter 2 of this study we investigated effects of trace elements and radionuclides on assemblage characteristics including the number of individuals collected (activity abundance), species diversity (from a rarefaction diversity index), and community composition of carrion-associated beetles. We collected scavenger and predator beetles from a total of 162 carrion traps placed at both contaminated and uncontaminated reference sites along a gradient from 0-300 m from the water's edge. Across all sites we captured 17,800 individual beetles representing 112 species in nine families, from which we looked at assemblage characteristics among the total assemblage, scavenging taxa, and predatory taxa. In Chapter 3, we used similar methodology to collect 113 individual Scarabaeidae and 51 individual Silphidae beetles to quantify contaminant body burdens in these taxa at two adjacent sites with coal combustion waste and a site with radiocesium from nuclear effluent, as well as an uncontaminated reference site.

Our results from Chapter 2 revealed variability within and among sites in activity abundance, species diversity, and species composition. Contrary to our predictions, overall there was higher activity abundance and diversity at contaminated locations, which was mainly driven by scavenger, as opposed to predatory, beetles. The variable of distance away from water added additional variability to the results. For example, activity abundance increased and decreased with increasing distance at the Ash Basin and Pond A respectively. There also was variation in activity abundance between the total assemblage, scavenger taxa, and predatory taxa, however, there were generally opposing responses between scavenger and predatory taxa. For example, at the Acid Pond/Steel Creek Bay site-pair, activity abundance of scavenger taxa decreased and predatory taxa increased at the contaminated site. Furthermore, across both contaminated and uncontaminated sites our data generally revealed differences in species composition between sampling locations immediately adjacent to the water's edge and more distant sampling locations. This could possibly demonstrate sensitivities of carrion-associated beetles to shifting habitat characteristics, such as percent overstory and understory cover as these conditions typically were much lower adjacent to water sources sampled in our study. For example, in open areas adjacent to the Ash Basin captures were dominated by species of the genus *Tetracha* (tiger beetles), which are open-habitat specialists, whereas at distances further from the water's edge at this site captures were dominated by forest interior specialists in the genus *Nicrophorus* (carrion beetles). *Nicrophorus* spp. were further identified as indicator species in forested sampling locations not adjacent to the water's edge at the Ash Basin, Acid Pond, Steel Creek Bay, and Thunder Bay.

Findings from this study suggest that contaminated ecosystems on the SRS have robust populations of carrion-associated beetles but there are clear differences in numbers between scavenger and predatory beetles that warrant further investigation. Nonetheless, in general traps at both contaminated and uncontaminated locations had high numbers of beetles. For example, 40-70% of traps at contaminated sites had trap counts of over 100 individual beetles, whereas 20-52% of traps at uncontaminated locations had counts over 100 individual beetles. In 186 traps from this study, there was never an instance of a trap collection that was absent of any carrion-associated beetles. Even though our study didn't find reduced activity abundance or diversity due to the presence of contamination, long-term global monitoring reveals that there is an overall decline in invertebrate abundance worldwide (Dirzo et al. 2014). When there is a decline in biodiversity characteristics, such as abundance and diversity, there is a generally a corresponding decline in the long-term functioning of ecosystem processes (Naeem et al. 1999). Our results suggest that habitat conditions may be more important in determining assemblage characteristics that contamination at the SRS.

Although findings for the assemblage of carrion-associated beetles were variable between site-pairs, differences in the accumulation of trace elements within beetle tissues were more explicit between contaminated and uncontaminated sites. Our results from Chapter 3 revealed variability in the bioaccumulation of contaminants among trace metals, between Scarabaeidae and Silphidae beetles, and between contaminated and uncontaminated sites. Overall we observed higher levels of As, Se, and Tl at the contaminated site, which was expected due to the presence of coal combustion waste at that location. The uncontaminated site (Steel Creek Bay) had higher levels of Cr, Cu, and Ni, which may reflect unique geochemical composition at this site. Silphidae beetles (scavengers) had higher levels of Chromium (Cr), Copper (Cu), Iron (Fe), Manganese (Mn), Nickel (Ni), and Zinc (Zn) compared to Scarabaeidae beetles (predators), which may reflect differences in trophic position and food preferences between the two groups. Variability in the concentration of trace elements also was variable among individuals of the same group, reflecting variability in bioaccumulation within the population, which may reflect differences in dietary selection. In our study, radiocesium was detected in elevated amounts (1.16 Bq/g and 1.4 Bq/g) in two only beetles, both from a site with known radiocesium contamination. Although these organisms are generally excluded from the human diet, these two individual beetles each had levels of radiocesium that exceed the European Economic Community limit of 0.600 Bq/g for meat consumption (EEC 1986) suggesting elevated levels compared to international standards.

The degree to which accumulation of contaminants affects the function of individual scavenger beetles at SRS is still unknown. Due to elevated levels of As, Se, and Tl in scavenging beetles from the contaminated location, it is likely that trace elements are transferred through trophic levels from vertebrate carrion to scavenging beetles. Additionally, our data suggest beetles at contaminated locations are pathways of contaminant transfer through the food chain, since they are a primary food source for some avian species (Bryan et al. 2012). Although this research demonstrated the ability of Coleopteran beetles to bioaccumulate radiocesium, the extent to which this occurs in landscapes with a more ubiquitous distribution of contamination (e.g., Chernobyl) and the rate of accumulation remains unknown. In addition, future research is needed to elucidate the effects of contaminant exposure on the health of beetles. For example, fluctuating asymmetry (differences in symmetry between the left and right sides) and increase in deformations has been used as a successful stress index for invertebrates (Polak et al. 2002).

From the individual to community level, invertebrates have variable responses to the presence of industrial contaminants, which often result in elevated stress for many organisms (Calow 1991, Forbes and Calow 1996). Chronic stress from elevated levels of contaminants requires defensive and repair processes which continuously divert energy resources away from fitness components such as growth and reproduction (Parsons 1991). Deterioration of fitness components at the individual level can extend to a reduction in population vitality, and eventually community composition (Hoffman and Parsons 1991). This study has provided a baseline inventory for the species composition of carrion associated beetles at the SRS in addition to element levels of Scarabaeidae and Silphidae, which are important monitoring tools to investigate assemblage shifts and stress levels that may expand into ecosystem functioning.

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APPENDICES

Appendix A. List of species collected from the site-pair Ash Basin (coal-fly ash)/Pond T (paired reference site) at the Savannah River Site, South Carolina in 2014.

Site	**Family**	**Species**		**Total Individuals**
Ash Basin	Carabidae	*Brachinus alternans*	Dejean	3
	Carabidae	*Carabus vinctus*	(Weber)	1
	Carabidae	*Chlaenius erythropus*	(Germar)	1
	Carabidae	*Cyclotrachelus laevipennis*	(Leconte)	1
	Carabidae	*Cyclotrachelus levifaber*	(Freitag)	6
	Carabidae	*Dicaelus dilatatus*	(Say)	10
	Carabidae	*Galerita janus*	(Fabricius)	13
	Carabidae	*Scarites subterraneus*	(Fabricius)	2
	Cincindelidae	*Tetracha carolina*	(Linnaeus)	9
	Cincindelidae	*Tetracha virginica*	(Linnaeus)	7
	Dermestidae	*Dermestes caninus*	Germar	3
	Geotrupidae	*Geotrupes blackburnii*	(Fabricius)	3
	Geotrupidae	*Geotrupes egeriei*	(Germar)	7
	Histeridae	*Euspilotus assimilis*	(Paykull)	1298
	Histeridae	*Hister abbreviatus*	(Fabricius)	1
	Histeridae	*Hister coenosus*	Erichson	136
	Scarabaeidae	*Aphodius rusicola*	(Melsheimer)	1
	Scarabaeidae	*Ateuchus lecontei*	Harold	13
	Scarabaeidae	*Canthon chalcites*	(Haldeman)	7
	Scarabaeidae	*Copris minutus*	(Drury)	89
	Scarabaeidae	*Deltochilum gibbosum*	Fabricius	499
	Scarabaeidae	*Onthophagus concinnus*	Laporte	4
	Scarabaeidae	*Onthophagus depressus*	(Harold)	5
	Scarabaeidae	*Onthophagus hecate hecate*	(Panzer)	403
	Scarabaeidae	*Onthophagus orpheus*	(Panzer)	3
	Scarabaeidae	*Onthophagus pennsylvanicus*	Harold	1
	Scarabaeidae	*Onthophagus striatulus*	(Beauvois)	65
	Scarabaeidae	*Onthophagus subaeneus*	(Beauvois)	1
	Scarabaeidae	*Onthophagus taurus*	(Schreber)	22
	Scarabaeidae	*Phanaeus igneus*	(MacLeay)	3
	Scarabaeidae	*Phanaeus triangularis*	(Say)	8
	Scarabaeidae	*Phanaeus vindex*	(MacLeay)	2
	Scarabaeidae	*Phyllophaga clemens*	(Horn)	2

	Silphidae	*Necrodes surinamensis*	Fabricius	41
	Silphidae	*Necrophila americana*	(Linnaeus)	33
	Silphidae	*Nicrophorus carolinus*	(Linnaeus)	15
	Silphidae	*Nicrophorus orbicollis*	Say	324
	Staphylinidae	*Creophilus maxilosus*	(Linnaeus)	205
	Staphylinidae	*Ontholestes cingulatus*	(Gravenhorst)	2
	Staphylinidae	*Platydracus comes*	(Leconte)	1
	Staphylinidae	*Platydracus femoratus*	(Fabricius)	1
	Staphylinidae	*Platydracus fossator*	(Gravenhorst)	1
	Staphylinidae	*Platydracus maculosus*	(Leconte)	89
	Trogidae	*Omorgus monachus*	(Herbst)	153
	Trogidae	*Omorgus suberosus*	(Fabricius)	1
	Trogidae	*Trox foveicollis*	Harold	14
	Trogidae	*Trox unistriatus*	Beauvois	26
Pond T	Carabidae	*Agonum albicrus*	(Dejean)	1
	Carabidae	*Anisodactylus rusticus*	(Say)	1
	Carabidae	*Apenes sinuatus*	(Say)	1
	Carabidae	*Brachinus alternans*	Dejean	5
	Carabidae	*Carabus vinctus*	(Weber)	2
	Carabidae	*Chlaenius emarginatus*	(Say)	4
	Carabidae	*Chlaenius erythropus*	(Germar)	4
	Carabidae	*Cyclotrachelus levifaber*	(Freitag)	8
	Carabidae	*Cyclotrachelus sigillatus*	(Say)	9
	Carabidae	*Dicaelus dilatatus*	(Say)	8
	Carabidae	*Dicaelus furvus*	Dejean	2
	Carabidae	*Galerita janus*	(Fabricius)	6
	Carabidae	*Mochtherus tetraspilotus*	(MacLeay)	1
	Carabidae	*Oodes amaroides*	(Dejean)	1
	Carabidae	*Pasimachus marginatus*	(Fabricius)	1
	Carabidae	*Scarites quadricepts*	(Chaudoir)	2
	Carabidae	*Stenocrepis mexicana*	(Chevrolat)	3
	Cincindelidae	*Tetracha carolina*	(Linnaeus)	1
	Cincindelidae	*Tetracha virginica*	(Linnaeus)	2
	Dytiscidae	*Copelatus glyphicus*	(Say)	1
	Geotrupidae	*Geotrupes blackburnii*	(Fabricius)	1
	Histeridae	*Euspilotus assimilis*	(Paykull)	1635
	Histeridae	*Hister abbreviatus*	(Fabricius)	1
	Histeridae	*Hister coenosus*	Erichson	101
	Scarabaeidae	*Ateuchus lecontei*	Harold	29
	Scarabaeidae	*Canthon chalcites*	(Haldeman	7
	Scarabaeidae	*Copris minutus*	(Drury)	107
	Scarabaeidae	*Deltochilum gibbosum*	Fabricius	327
	Scarabaeidae	*Melanocanthon bispinatus*	(Robinson)	5

Scarabaeidae	*Onthophagus concinnus*	Laporte	5
Scarabaeidae	*Onthophagus hecate hecate*	(Panzer)	136
Scarabaeidae	*Onthophagus striatulus*	(Beauvois)	45
Scarabaeidae	*Onthophagus taurus*	(Schreber)	12
Scarabaeidae	*Phanaeus igneus*	(MacLeay)	2
Scarabaeidae	*Phanaeus triangularis*	(Say)	1
Scarabaeidae	*Phileurus valgus*	(Linnaeus)	1
Scarabaeidae	*Pseudocanthon perplexus*	(LeConte)	4
Silphidae	*Necrodes surinamensis*	Fabricius	11
Silphidae	*Necrophila americana*	(Linnaeus)	28
Silphidae	*Nicrophorus carolinus*	(Linnaeus)	6
Silphidae	*Nicrophorus orbicollis*	Say	77
Silphidae	*Creophilus maxilosus*	(Linnaeus)	35
Staphylinidae	*Platydracus cinnamopterus*	(Gravenhorst)	1
Staphylinidae	*Platydracus comes*	(Leconte)	2
Staphylinidae	*Platydracus maculosus*	(Leconte)	34
Trogidae	*Omorgus monachus*	(Herbst)	140
Trogidae	*Trox foveicollis*	Harold	66
Trogidae	*Trox terrestris*	Say	8

Appendix B. List of species collected at the site-pair Acid Pond (coal pile run-off)/Steel Creek Bay (paired reference site) at the Savannah River Site, South Carolina in 2015.

Site	**Family**	**Species**		**Total Individuals**
Acid	Carabidae	*Chlaenius emarginatus*	(Say)	3
Pond	Carabidae	*Cyclotrachelus levifaber*	(Freitag)	2
	Carabidae	*Dicaelus dilatatus*	(Say)	2
	Carabidae	*Dicaelus furvus*	Dejean	1
	Carabidae	*Galerita janus*	(Fabricius)	9
	Carabidae	*Harpalus sps.*		1
	Carabidae	*Scarites quadricepts*	(Chaudoir)	1
	Cincindelidae	*Cicindela sexguttata*	(Fabricius)	3
	Cincindelidae	*Tetracha carolina*	(Linnaeus)	2
	Dermestidae	*Dermestes caninus*	Germar	3
	Geotrupidae	*Geotrupes egeriei*	(Germar)	1
	Histeridae	*Euspilotus assimilis*	(Paykull)	335
	Histeridae	*Hister coenosus*	Erichson	103
	Histeridae	*Saprinus pennsylvanicus*	(Paykull)	1
	Scarabaeidae	*Ateuchus lecontei*	Harold	422
	Scarabaeidae	*Canthon chalcites*	(Haldeman	113
	Scarabaeidae	*Copris minutus*	(Drury)	14
	Scarabaeidae	*Deltochilum gibbosum*	Fabricius	784
	Scarabaeidae	*Dichotomius carolinus*	(Linnaeus)	1
	Scarabaeidae	*Euetheola humilis*	(Burmesiter)	1
	Scarabaeidae	*Hybosorus illigeri*	(Erichson)	5
	Scarabaeidae	*Melanocanthon bispinatus*	(Robinson)	58
	Scarabaeidae	*Onthophagus concinnus*	Laporte	145
	Scarabaeidae	*Onthophagus depressus*	(Harold)	12
	Scarabaeidae	*Onthophagus hecate hecate*	(Panzer)	282
	Scarabaeidae	*Onthophagus pennsylvanicus*	Harold	89
	Scarabaeidae	*Onthophagus striatulus*	(Beauvois)	9
	Scarabaeidae	*Onthophagus subaeneus*	Beauvois)	4
	Scarabaeidae	*Onthophagus taurus*	(Schreber)	243
	Scarabaeidae	*Onthophagus tuberculatus*	(Harold)	60
	Scarabaeidae	*Phanaeus igneus*	(MacLeay)	2
	Scarabaeidae	*Phanaeus triangularis*	(Say)	2

	Scarabaeidae	*Phanaeus vindex*	(MacLeay)	3
	Scarabaeidae	unidentified	-	2
	Scarabaeidae	unidentified	-	1
	Scarabaeidae	unidentified	-	1
	Silphidae	*Necrophila americana*	(Linnaeus)	147
	Silphidae	*Necrodes surinamensis*	Fabricius	79
	Silphidae	*Nicrophorus carolinus*	(Linnaeus)	33
	Silphidae	*Nicrophorus orbicollis*	Say	391
	Silphidae	*Nicrophorus pustulatus*	(Herschel)	7
	Silphidae	*Nicrophorus tormentosus*	(Weber)	40
	Silphidae	*Oiceoptoma inaequalis*	(Fabricius)	8
	Staphylinidae	*Aleochara sps.*	-	1
	Staphylinidae	*Creophilus maxilosus*	(Linnaeus)	98
	Staphylinidae	*Ontholestes cingulatus*	(Gravenhorst)	2
	Staphylinidae	*Platydracus comes*	(Leconte)	138
	Staphylinidae	*Platydracus fossator*	(Gravenhorst)	34
	Staphylinidae	*Platydracus maculosus*	(Leconte)	27
	Staphylinidae	unidentified	-	1
	Staphylinidae	unidentified	-	2
	Staphylinidae	*Tachinus fimbriatus*	(Gravenhorst)	4
	Trogidae	*Omorgus monachus*	(Herbst)	96
	Trogidae	*Omorgus suberosus*	(Fabricius)	1
	Trogidae	*Trox aequalis*	Say	1
	Trogidae	*Trox foveicollis*	Harold	37
	Trogidae	*Trox terrestris*	Say	23
	Trogidae	*Trox unistriatus*	Beauvois	12
Steel Creek Bay	Carabidae	*Carabus vinctus*	(Weber)	4
	Carabidae	*Cyclotrachelus levifaber*	(Freitag)	15
	Carabidae	*Cyclotrachelus sigillatus*	(Say)	9
	Carabidae	*Dicaelus dilatatus*	(Say)	1
	Carabidae	*Galerita janus*	(Fabricius)	3
	Carabidae	*Helluomorphoides nigripennis*	(Dejean)	2
	Geotrupidae	*Geotrupes blackburnii*	(Fabricius)	1
	Histeridae	*Euspilotus assimilis*	(Paykull)	357
	Histeridae	*Hister abbreviatus*	(Fab.)	16
	Histeridae	*Hister coenosus*	Erichson	54
	Histeridae	*Saprinus pennsylavanicus*	(Paykull)	2
	Scarabaeidae	*Ateuchus lecontei*	Harold	674
	Scarabaeidae	*Canthon chalcites*	(Haldeman	95
	Scarabaeidae	*Copris minutus*	(Drury)	15

Scarabaeidae	*Deltochilum gibbosum*	Fabricius	835
Scarabaeidae	*Diplotaxis punctatorugosa*	(Blanchard)	1
Scarabaeidae	*Melanocanthon bispinatus*	(Robinson)	17
Scarabaeidae	*Onthophagus concinnus*	Laporte	33
Scarabaeidae	*Onthophagus depressus*	(Harold)	2
Scarabaeidae	*Onthophagus hecate hecate*	(Panzer)	49
Scarabaeidae	*Onthophagus pennsylvanicus*	Harold	46
Scarabaeidae	*Onthophagus striatulus*	(Beauvois)	7
Scarabaeidae	*Onthophagus subaeneus*	(Beauvois)	1
Scarabaeidae	*Onthophagus taurus*	(Schreber)	27
Scarabaeidae	*Onthophagus tubercalatus*	(Harold)	10
Scarabaeidae	*Phanaeus igneus*	(MacLeay)	4
Scarabaeidae	*Phanaeus vindex*	(MacLeay)	5
Scarabaeidae	*Phyllophaga forsteri*	(Burmeister)	1
Scarabaeidae	*Phyllophaga ilicis*	(Knoch)	4
Scarabaeidae	*Phyllophaga clemens*	(Horn)	1
Silphidae	*Necrophila americana*	(Linnaeus)	134
Silphidae	*Necrodes surinamensis*	Fabricius	19
Silphidae	*Nicrophorus carolinus*	(Linnaeus)	35
Silphidae	*Nicrophorus orbicollis*	Say	280
Silphidae	*Nicrophorus pustulatus*	(Herschel)	4
Silphidae	*Nicrophorus tormentosus*	(Weber)	32
Staphylinidae	*Creophilus maxilosus*	(Linnaeus)	93
Staphylinidae	*Philonthus umbrinus*	(Gravenhorst)	2
Staphylinidae	*Platydracus comes*	(Leconte)	203
Staphylinidae	*Platydracus femoratus*	(Fabricius)	8
Staphylinidae	*Platydracus fossator*	(Gravenhorst)	33
Staphylinidae	*Platydracus maculosus*	(Leconte)	40
Trogidae	*Omorgus monachus*	(Herbst)	72
Trogidae		(Fabricius)	1
Trogidae	*Trox aequalis*	Say	1
Trogidae	*Trox foveicollis*	Harold	5
Trogidae	*Trox tuberculatus*	De Geer	1

Appendix C. List of species collected at site-pair Pond A (radiocesium)/ Thunder Bay (paired references site) at the Savannah River Site, South Carolina in 2015.

Site	Family	Species		Total Individuals
Pond A	Carabidae	*Anisodactylus rusticus*	(Say)	1
	Carabidae	*Brachinus alternans*	Dejean	8
	Carabidae	*Calathus opaculus*	LeConte	4
	Carabidae	*Carabus goryi*	Dejean	1
	Carabidae	*Carabus sylvosus*	Say	2
	Carabidae	*Carabus vinctus*	(Weber)	1
	Carabidae	*Cyclotrachelus sigillatus*	(Say)	6
	Carabidae	*Dicaelus furvus*	Dejean	5
	Carabidae	*Dicaelus purpuratus*	Bonelli	1
	Carabidae	*Pasimachus subsulcatus*	Say	1
	Cincindelidae	*Tetracha carolina*	(Linnaeus)	1
	Dermestidae	*Dermestes caninus*	Germar	2
				18
	Histeridae	*Euspilotus assimilis*	(Paykull)	8
	Histeridae	*Hister coenosus*	Erichson	29
	Histeridae	*Hister fungicola*	Schaeffer	1
	Scarabaeidae	unidentified	-	2
				16
	Scarabaeidae	*Ateuchus lecontei*	Harold	2
	Scarabaeidae	*Canthon chalcites*	(Haldeman)	11
	Scarabaeidae	*Copris minutus*	(Drury)	4
				19
	Scarabaeidae	*Deltochilum gibbosum*	Fabricius	4
	Scarabaeidae	*Diplotaxis punctatorugosa*	(Blanchard)	1
	Scarabaeidae	*Melanocanthon bispinatus*	(Robinson)	42
	Scarabaeidae	*Onthophagus concinnus*	Laporte	37
				50
	Scarabaeidae	*Onthophagus hecate hecate*	(Panzer)	6
	Scarabaeidae	*Onthophagus pennsylvanicus*	Harold	48
	Scarabaeidae	*Onthophagus striatulus*	(Beauvois)	5
	Scarabaeidae	*Onthophagus tuberculatus*	(Harold)	31
	Silphidae	*Necrodes surinamensis*	Fabricius	5
	Silphidae	*Necrophila americana*	(Linnaeus)	26
	Silphidae	*Nicrophorus carolinus*	(Linnaeus)	1
	Silphidae	*Nicrophorus orbicollis*	Say	52
	Silphidae	*Nicrophorus tomentosus*	Weber	39

	Silphidae	*Oiceoptoma inaequalis*	(Fabricius)	12
	Staphylinidae	unidentified	-	1
	Staphylinidae	*Aleochara sps.*	-	2
	Staphylinidae	*Platydracus comes*	(Leconte)	2
	Staphylinidae	*Platydracus maculosus*	(Leconte)	6
	Staphylinidae	*Platydracus praetermissus*	(Newton)	2
	Staphylinidae	*Tachinus fimbriatus*	Gravenhorst	31
	Trogidae	*Omorgus monachus*	(Herbst)	9
	Trogidae	*Trox foveicollis*	Harold	70
	Trogidae	*Trox terrestris*	Say	5
Thunder	Carabidae	*Anisodactylus rusticus*	(Say)	2
Bay	Carabidae	*Calathus opaculus*	LeConte	1
	Carabidae	*Carabus sylvosus*	Say	6
	Carabidae	*Carabus vinctus*	(Weber)	10
	Carabidae	*Dicaelus furvus*	Dejean	1
	Carabidae	*Dicaelus purpuratus*	Bonelli	1
	Carabidae	*Helluomorphoides clairvillei*	(Dejean)	1
	Carabidae	*Helluomorphoides nigripennis*	(Dejean)	1
	Dermestidae	*Dermestes caninus*	Germar	3
	Geotrupidae	*Bolboceras thoracicornis*	(Wallis)	1
	Histeridae	*Euspilotus assimilis*	(Paykull)	74 3
	Histeridae	*Hister coenosus*	Erichson	20 9
	Histeridae	*Onthophilus nodatus*	LeConte	1
	Histeridae	*Saprinus lugens*	Erichson	2
	Scarabaeidae	unidentified	-	1
	Scarabaeidae	unidentified	-	3
	Scarabaeidae	unidentified	-	1
	Scarabaeidae	unidentified	-	1
	Scarabaeidae	*Canthon chalcites*	(Haldeman)	18
	Scarabaeidae	*Copris minutus*	(Drury)	23
	Scarabaeidae	*Deltochilum gibbosum*	Fabricius	18 4
	Scarabaeidae	*Digitonthophagus gazella*	(Fabricius)	1
	Scarabaeidae	*Melanocanthon bispinatus*	Robinson	27
	Scarabaeidae	*Onthophagus concinnus*	Laporte	39
	Scarabaeidae	*Onthophagus hecate hecate*	(Panzer)	72
	Scarabaeidae	*Onthophagus pennsylvanicus*	Harold	99
	Scarabaeidae	*Onthophagus striatulus*	(Beauvois)	13
	Scarabaeidae	*Onthophagus taurus*	(Schreber)	12
	Scarabaeidae	*Onthophagus tubercalatus*	(Harold)	54
	Scarabaeidae	*Phanaeus igneus*	MacLeay	3

			49
Scarabaeidae	*Ateuchus lecontei*	Harold	4
Silphidae	*Necrodes surinamensis*	Fabricius	21
Silphidae	*Necrophila americana*	(Linnaeus)	62
Silphidae	*Nicrophorus carolinus*	(Linnaeus)	3
			10
Silphidae	*Nicrophorus orbicollis*	Say	3
Silphidae	*Nicrophorus pustulatus*	Hercshal	6
Silphidae	*Nicrophorus tormentosus*	Weber	85
Staphylinidae	unidentified	-	1
Staphylinidae	unidentified	-	1
Staphylinidae	unidentified	-	1
Staphylinidae	unidentified	-	1
Staphylinidae	unidentified	-	2
Staphylinidae	*Aleochara sps.*	-	2
			15
Staphylinidae	*Creophilus maxilosus*	(Linnaeus)	9
Staphylinidae	*Ontholestes cingulatus*	(Gravenhorst)	22
Staphylinidae	*Platydracus comes*	(Leconte)	9
Staphylinidae	*Platydracus maculosus*	(Leconte)	20
Staphylinidae	*Tachinus fimbriatus*	Gravenhorst	2
Trogidae	*Omorgus monachus*	(Herbst)	23
Trogidae	*Trox foveicollis*	Harold	69
Trogidae	*Trox tuberculatus*	De Geer	1
Trogidae	*Trox unistriatus*	Beauvois	1