PASSERINE COMMUNITIES INHABITING COAL COMBUSTION AND NUCLEAR FISSION WASTE SITES: QUANTIFYING MULTISCALE VARIANCE IN CONTAMINANT UPTAKE AND EFFECTS

by

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(Under the Direction of Travis L. DeVault and Olin E. Rhodes Jr.)

ABSTRACT

The release of coal combustion residuals and nuclear fission products creates contaminated landscapes where resident wildlife are exposed to excess metals, metalloids, and radionuclides. The threat that these contaminants pose to wildlife can be difficult to characterize given their diverse physiological and environmental fates and varying toxicities. We use a multiscale approach to address variation in contaminant uptake and sublethal effects among the passerine bird community inhabiting coal combustion and nuclear fission legacy waste areas on the Department of Energy's Savannah River Site. We 1) investigated how contaminant uptake varies with habit use strategies among the passerine community, 2) examined the effects of contamination on the dynamics of passerine hosts, dipteran vectors, and haemosporidian parasites, and 3) used acoustic indices to evaluate shifts in dawn and dusk chorus activity across contaminated landscapes. The transfer of waste-derived contaminants to passerines varied with species traits and is associated with nuanced disruptions to their ecological roles. Our findings provide insight into the behavior of waste-derived contaminants in the environment, their impacts on sensitive wildlife, and their cascading effects on ecosystems.

INDEX WORDS: coal combustion residuals; disease ecology; ecoacoustics; ecotoxicology; nuclear fission products; parasitism; remediation; soundscape ecology

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Mismanagement of industrial waste has led to the dissemination of heavy metals, metalloids, and radionuclides in natural environments worldwide, with consequences for the health of humans, wildlife, and their shared ecosystems (Alloway, 1995, Jiang et al., 2022, Kapustka, 2004). Within individual organisms, exposure to excess trace element concentrations or ionizing radiation can affect critical cellular processes, with consequences for growth, reproduction, immune function, and behavior (Klerks and Levinton, 1989, Roy et al., 2015, Warren and Klaine, 1995). At the population, community, and ecosystem levels, dynamic ecological processes such as adaptation, species interactions, and biogeochemical cycling complicate the environmental fates and toxicity effects of trace element contaminants (Kapustka, 2004, Sullivan and Rodewald, 2012).

The development of nuclear weapons in the mid-20th century was accompanied by a legacy of intense and far-reaching environmental impact (Hu et al., 2010). Between 1945 and 1980, over 500 atmospheric weapons tests dispersed cesium-137, strontium-90, plutonium-239, and other radionuclides across the surface of the Earth (Choppin, 2007, UNSCEAR, 2000, Waters et al., 2015). On a local scale, the manufacture of fissile uranium, plutonium, and tritium outpaced the implementation of proper waste disposal, resulting in significant releases of nuclear fission products (NFP) to the environments surrounding production facilities (Bradley et al., 1996). In the United States, localized NFP contamination occurred on several, large tracts of land owned by the US Department of Energy, including the Hanford Site in Washington (1,518 km^2), the Oak Ridge Reservation in Tennessee (135 km^2), and the Savannah River Site in South Carolina (780 km^2; Burger and Carletta, 2004, Dwivedi et al., 2022). The production of fissile materials at these sites relied heavily on energy supplied by coal combustion facilities, which generated their own metal, metalloid, and radionuclide byproducts. Coal combustion residuals (CCR) were stored in open impoundments that were subjected to leaching, spillover, or collapse (Denham and Nichols, 1995, Ruhl et al., 2009, Sulloway, 2012). The advent of key environmental legislation in the late 20^{th} century prompted former nuclear production sites to prioritize environmental remediation and waste management, but the excavation of dispersed legacy contamination was often unfeasible or cost-prohibitive (Burger and Carletta, 2004, Dwivedi et al., 2022). Therefore, waste-derived radionuclides, metals, and metalloids remain in the environment at former nuclear production sites, where they present long-term challenges for land stewardship.

Despite their legacies of contamination, federal lands affected by nuclear legacy wastes often contain valuable ecological resources, including rare habitats and threatened or endangered wildlife (Burger and Carletta, 2004). Due to the localized nature of NFP or CCR contamination, such landscapes represent mosaics of contaminated areas interspersed with higher-quality, minimally-impacted habitat (Carlsen et al., 2004). Attractive habitat features such as constructed wetlands, combined with limited human activity and industrial development, can recruit wildlife to contaminated lands from surrounding habitat patches (Coates, 2013, Sievers et al., 2018). The colonization of legacy waste sites by wildlife can lead to the integration of waste-derived elements into dynamic ecological processes that enhance their bioavailability, alter their environmental fates, and affect wildlife health or ecosystem function (Avery, 1996, Brandt et al., 2019, Klerks and Levinton, 1989, Silva et al., 2023). Understanding the bioavailability and accumulation risks of trace elements in wildlife is crucial for implementation of environmental remediation efforts on contaminated sites.

Passerine birds are susceptible to metal, metalloid, and radionuclide accumulation via dietary uptake or contact with contaminated substrates, water, and air (Burger, 1993, Einoder et al., 2018, Scheuhammer, 1987). Studies of contaminant toxicity in various taxa, including birds, gained popularity with the passage of several foundational pieces of environmental legislation, notably the Clean Water Act of 1970 (Kapustka, 2004). Early toxicologists designed experiments that focused on exposing model organisms to varying doses of heavy metals, metalloids, and radionuclides in laboratory settings. Experiments conducted on birds revealed a variety of toxicity effects to multiple organ systems (Scheuhammer, 1987). For example, adult mallard ducks fed a diet containing 200 ppm of cadmium experienced suppressed egg production (White & Finley, 1978) and kidney failure within 60 days (White et al. 1978), and chickens fed 250 ppm of mercury showed stunted growth (Parkhurst and Thaxton, 1973). Low doses of methyl-mercury hindered reproductive success and caused spinal cord lesions in pheasants (Borg et al. 1969), and starlings fed a diet of 90 ppm lead exhibited decreased brain weights (Grue et al. 1986). These early experiments revealed physiological mechanisms by which contaminants are incorporated into tissues and established baseline dose-response thresholds for toxicity in birds.

Although dose-response thresholds are useful tools for evaluating potential risks of contaminant exposure, the effects observed in laboratory settings cannot always be extrapolated to field conditions. For example, the absorption rates of heavy metals can vary widely with the trophic guilds and habitat preferences of different bird species, and birds occupying the same contaminated sites can carry very different burdens of heavy metals (Abbasi et al., 2015). Some contaminants bioaccumulate in the tissues of prey organisms, increasing rates of exposure for birds at higher trophic levels (Abbasi et al., 2015, Leonzio et al., 2009). Differences in trophic guilds coincide with a five-fold difference in mercury concentrations measured in seabird eggs (Burger, 2002). Obligate insectivores, carnivores, and piscivores foraging in freshwater systems have higher dietary uptake if pollutants are absorbed and retained by prey organisms throughout their life cycles (Leonzio et al., 2009, Sullivan and Rodewald, 2012).

Risk of contaminant uptake for various bird species can also depend on the properties of trace elements. For example, ^{137}Cs readily binds to sediments, and birds occupying lower trophic levels may be at higher risk for ^{137}Cs uptake from contaminated soil or plants (Avery, 1996). This pattern was demonstrated in ecosystems surrounding the Fukushima-Daiichi nuclear power plant, where invertebrates that forage on decaying plant matter had higher ^{137}Cs activity than carnivorous invertebrates (Ishii et al., 2017). At the Savannah River Site, adult bullfrogs had lower ^{137}Cs than tadpoles despite having more carnivorous diets

(Leaphart et al., 2020). Variation in the transfer of NFP-derived radionuclides to passerines has been demonstrated within the Chernobyl and Fukushima exclusion zones and in remote locations affected by fallout from these accidents (Beresford et al., 2016, Baeza et al., 1991, Lonvik and Thingstad, 1999, Krivolutski et al., 1999, Sternalski et al., 2015). However, many field studies of avian communities in polluted environments have focused on a few or even a single species as bioindicators of contamination (Dmowski, 1999, Lin et al., 2021), which limits their conclusions on the dietary or behavioral correlates of uptake. Efforts to understand contaminant uptake patterns in songbirds must account for the wide variation in trace element properties and bioavailability by investigating multiple species across sites with different contamination histories.

Another limitation of traditional, laboratory-derived toxicity experiments is their exclusion of the effects of ecologically relevant stressors that can exacerbate the impacts of contaminant uptake in wildlife, such as parasitism (Bichet et al., 2013). Avian *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* parasites are common haemosporidian blood parasites of passerine birds that are transmitted via dipteran vectors (Kimura et al., 2010, Paul et al., 2003, Santiago-Alarcon et al., 2012). Once transmission occurs, haemosporidians form a chronic infections in avian hosts, which then continue to infect new vectors throughout their lives (Valkiunas, 2004). Avian malaria, the disease caused by *Plasmodium,* induces stress in birds and has played a significant role in the decline of vulnerable bird populations (Atkinson and Samuel, 2010).

Contaminants may exacerbate malaria in wild birds by increasing host susceptibility to infection. Evidence suggests that high concentrations of cadmium and mercury are associated with suppressed immune function in birds (Henry et al., 2014, Koller, 1980). Heavy metals can also increase oxidative stress, which further hinders immune response (Wada and Coutts, 2021). Alternatively, low concentrations of essential trace elements, such as copper, may stimulate immune function due to a hormetic effect (Kapustka, 2004, Wada and Coutts, 2021), in which case birds living in habitats with low levels of contaminants may demonstrate increased resistance to haemosporidian parasites.

In addition to effects on host susceptibility, contaminants may alter the abundance of vectors or parasites in polluted ecosystems, thereby changing rates of host exposure (Neff and Dharmarajan, 2021, Wada and Coutts, 2021). A study at the Savannah River Site found an inverse relationship between ^{137}Cs and *Hepatozoon* prevalence in Florida green watersnakes, suggesting that ^{137}Cs could disrupt the life cycle of the parasite (Brown et al., 2022). Reduced vector survival in contaminated ecosystems would lower host-vector contact rates, which could decrease prevalence of vector-borne parasites even if hosts are immunosuppressed. Laboratory experiments have demonstrated reduced larval survival of *Anopheles, Aedes,* and *Culex* mosquitoes exposed to solutions of cadmium, copper, and lead (Amer et al., 2021, Mireji et al., 2010, Neff and Dharmarajan, 2021). However, most mosquito toxicity experiments are conducted on naïve laboratory populations, whereas mosquitoes in field conditions might adapt to the presence of metals (Mireji et al., 2010). In addition, one study found that very low concentrations of copper increased fecundity of *Aedes aegypti,* consistent with the hormetic effect of essential trace elements (Neff and Dharmarajan, 2021). Competing mechanisms could contribute to the overall effect of contaminants on haemosporidian parasites in birds, and field studies must be carefully designed to disentangle these complex dynamics.

The costs of contaminant uptake in songbirds can extend beyond direct toxicity effects on survival and reproduction to include more subtle effects on behavior that indirectly reduce fitness (Kapustka, 2004). For instance, toxicity-induced cognitive deficiencies or lower energy budgets can alter complex social behaviors that are important for mate selection and reproductive success. Male acoustic communication is one energetically expensive behavior that can be compromised by exposure to contaminants (Gorissen et al., 2005, Hallinger et al., 2010). Birdsong requires the coordination of cognitive, respiratory, and motor functions (Logue et al., 2020). Sexual dimorphism in birdsong is a product of sexual selection, and therefore male acoustic performance is expected to serve as an honest indicator of male quality (Sung and Handford, 2020). Environmental stressors, including heavy metal contamination, can induce sublethal effects in birds that manifest through subtle changes in behavior such as acoustic performance (Casagrande et al., 2016).

Prior studies indicate that male songbirds on polluted sites might exhibit reduced song performance. Male great tits (*Parus major*) inhabiting a smelter site heavily polluted with lead, arsenic, copper, cadmium, and zinc spent less time singing and had a reduced repertoire size compared with males from an unpolluted site (Gorissen et al., 2005). The songs of male Carolina wrens (*Thryothorus ludovicianus*), house wrens (*Troglodytes aedon*), and song sparrows (*Melospiza melodia*) on mercury-polluted sites were less diverse and had lower bandwidth than those from unpolluted sites (Hallinger et al., 2010). The "developmental stress hypothesis" proposes that nestlings exposed to pollutants early in life can experience reduced capacity for song learning, resulting in a smaller repertoire (Nowicki et al., 1998). Additionally, metals may alter the physiological structures required for producing songs. In black-spotted frogs (*Pelophylax nigromaculata*), treatment with copper resulted in reduced size of the larynx in males, which hindered the physical capacity to sing (Duan and Huang, 2016).

Birdsong often dominates the unique acoustic signature of landscapes, especially during the breeding season and at dawn (Staicer et al., 1996). Advances in the field of soundscape ecology have led to the development of acoustic indices that can detect shifts in soundscapes due to environmental change (Pijanowski et al., 2011, Pijanowski, 2024). Acoustic indices have been used to estimate avian species richness (Towsey et al., 2014, Gasc et al., 2013), investigate songbird phenology (Buxton et al., 2016, Morales et al., 2022), and compare dawn chorus activity across heterogenous landscapes (Farina et al., 2015). However, many potential applications of soundscape ecology methods to ornithology remain unexplored, including the development of metrics for avian response to human disturbance (Gasc et al., 2017). Additionally, acoustic indices have the potential to complement biomonitoring initiatives in contaminated habitat by providing an efficient means to measure the broader, acoustically-active community at larger spatial and temporal scales.

Understanding the multiple ways trace element contaminants affect avian communities is essential for protecting passerines in contaminated habitats. This study transcends reliance on simplistic dose-response thresholds by investigating how metal, metalloid, and radionuclide contaminants are incorporated into multiple ecological processes relevant to the avian community. In the following chapters, we present three studies that evaluate variation in contaminant uptake and effects among passerine birds and their broader ecological communities. In Chapter 2, we evaluate how differences in habitat use predict burdens of waste-derived metals, metalloids, and radionuclides across passerine species inhabiting legacy waste sites. In Chapter 3, we investigate the effects of contaminants on the dynamics of haemosporidian parasites in passerine hosts and dipteran vectors. Finally, in Chapter 4, we evaluate changes in the dawn chorus soundscape across landscapes with varying contaminant impacts. Collectively, these studies provide an innovative and multi-scale ecological risk assessment that contributes to our understanding of contaminant impacts on wildlife.

LITERATURE CITED

Abbasi, N. A., Jaspers, V. L. B., Chaudhry, M. J. I., Ali, S. & Malik, R. N. 2015. Influence of taxa, trophic level, and location on bioaccumulation of toxic metals in bird's feathers: a preliminary biomonitoring study using multiple bird species from Pakistan. *Chemosphere,* 120**,** 527-537. https://doi.org/10.1016/j.chemosphere.2014.08.054

Alloway, b. J. 1995. *Metals in soils,* New York, Blackie Academic & Professional, Chapman & Hall.

Amer, N. R., Lawler, S. P., Zohdy, N. M., Younes, A., ElSayed, W. M. & Connon, R. E. 2021. Effect of long-term exposure to copper on survival and development of two successive generations of *Culex pipiens*. *Ecotoxicology,* 30**,** 351-360.

Atkinson, C. T. & Samuel, M. D. 2010. Avian malaria Plasmodium relictum in native Hawaiian forest birds: Epizootiology and demographic impacts on 'apapane Himatione sanguinea. *Journal of Avian Biology,* 41**,** 357-366.

Avery, S. V. 1996. Fate of caesium in the environment: distribution between abiotic and biotic components of aquatic and terrestrial ecosystems. *Journal of Environmental Radioactivity,* 30**,** 139-171. https://doi.org/10.1016/0265-931X(96)89276-9

Baeza, A., del Rio, M., Miro, C., Moreno, A., Navarro, E., Paniagua, J. M. & Peris, M. A. 1991. Radiocesium and radiostrontium levels in song thrushes (*Turdus philomelos*) captured in two regions of Spain. *Journal of Environmental Radioactivity,* 13**,** 13-23. https://doi.org/10.1016/0265-931X(91)90036-F

Beresford, N. A., Gaschak, S., Maksimenko, A. & Wood, M. D. 2016. The transfer of ^{137}Cs, Pu isotopes, and ^{90}Sr to bird, bat and ground-dwelling small mammal species within the Chernobyl exclusion zone. *Journal of Environmental Radioactivity,* 153**,** 231-236. https://doi.org/10.1016/j.jenvrad.2015.12.027

Bichet, C., Scheifler, R., Cœurdassier, M., Julliard, R., Sorci, G. & Loiseau, C. 2013. Urbanization, Trace Metal Pollution, and Malaria Prevalence in the House Sparrow. *PLoS ONE,* 8**,** e53866. 10.1371/journal.pone.0053866

Bradley, D. J., Frank, C. W. & Mikerin, Y. 1996. Nuclear contamination from weapons complexes in the former Soviet Union and the United States. *Physics Today,* 49**,** 40-45. https://doi.org/10.1063/1.881495
Brandt, J. E., Simonin, M., Di Giulio, R. T. & Bernhardt, E. S. 2019. Beyond selenium: Coal combustion residuals lead to multielement enrichment in receiving lake food webs. *Environmental Science and Technology,* 53**,** 4119-4127. https://doi.org/10.1021/acs.est.9b00324
Brown, M. K., Haskins, D. L., Russell, A. L., Lambert, M. L., Quick, C. E., Pilgrim, M. A. & Tuberville, T. D. 2022. Mercury and Radiocesium Accumulation and Associations With Sublethal Endpoints in the Florida Green Watersnake (*Nerodia floridana*). *Environmental Toxicology and Chemistry,* 41**,** 758-770.
Burger, J. 1993. Metals in avian feathers: bioindicators of environmental pollution. *Reviews of Environmental Contamination and Toxicology***,** 203-311.
Burger, J. 2002. Food Chain Differences Affect Heavy Metals in Bird Eggs in Barbegat Bay, New Jersey. *Environmental Research,* 90**,** 33-39.
Burger, J. & Carletta, M. A. 2004. Assessing Ecological Resources for Remediation and Future Land Uses on Contaminated Lands. *Environmental Management,* 34**,** 1-10. https://www.doi.org/10.1007/s00267-004-0212-9
Buxton, R. T., Brown, E., Sharman, L., Gabriele, C. M. & McKenna, M. F. 2016. Using bioacoustics to examine shifts in songbird phenology. *Ecology and Evolution,* 6**,** 4697-4710.
Carlsen, T. M., Coty, J. D. & Kercher, J. R. 2004. The spatial extent of contamination and the landscape scale: an analysis of the wildlife, conservation biology, and population modeling literature. *Environmental Toxicology and Chemistry,* 23**,** 798-811. https://doi.org/10.1897/02-202
Casagrande, S., Pinxten, R. & Eens, M. 2016. Honest Signaling and Oxidative Stress: The Special Case of Avian Acoustic Communication. *Frontiers in Ecology and Evolution,* 4. https://doi.org/10.3389/fevo.2016.00052
Choppin 2007. Actinide speciation in the environment. *Journal of Radioanalytical and Nuclear Chemistry,* 273**,** 695-703. https://doi.org/10.1007/s10967-007-0933-3
Coates, P. 2013. From hazard to habitat (or hazardous habitat): The lively and lethal afterlife of Rocky Flats, Colorado. *Progress in Physical Geography: Earth and Environment,* 38. https://doi.org/10.1177/030913331351329
Denham, M. E. & Nichols, R. L. 1995. The speciation of groundwater contaminated with coal pile leachate at the Savannah River Site, South Carolina. Westinghouse Savannah River Company.
Dmowski, K. 1999. Birds as bioindicators of heavy metal pollution: review and examples concerning European species. *Acta Ornithologica,* 34**,** 1-25.
Duan, R.-Y. & Huang, M.-Y. 2016. The influence of low-dose cadmium on the laryngeal microstructure and ultrastructure of *Pelophylax nigromaculata*. *Environmental Science and Pollution Research,* 23**,** 17322-17331.
Dwivedi, D., Steefel, C. I., Arora, B., Banfield, J., Bargar, J., Boyanov, M. I., Brooks, S. C., Chen, X., Hubbard, S. S., Kaplan, D., Kemner, K. M., Nico, P. S., O'Loughlin, E. J., Pierce, E. M., Painter, S. L., Scheibe, T. D., Wainwright, H. M., Williams, K. H. & Zavarin, M. 2022. From legacy contamination to watershed systems science: a review of scientific insights and technologies developed through DOE-supported research in water and energy security. *Environmental Research,* 17. https://doi.org/10.1088/1748-9326/ac59a9
Einoder, L. D., MacLeod, C. K. & Coughanowr, C. 2018. Metal and Isotope Analysis of Bird Feathers in a Contaminated Estuary Reveals Bioaccumulation, Biomagnification, and Potential Toxic Effects. *Archives of Environmental Contamination and Toxicology,* 75**,** 96-110. https://doi.org/10.1007/s00244-018-0532-z
Farina, A., Ceraulo, M., Bobryk, C., Pieretti, N., Quinci, E. & Lattanzi, E. 2015. Spatial and temporal variation of bird dawn chorus and successive acoustic morning activity in a Mediterranean landscape. *Bioacoustics,* 24**,** 269-288.

Gasc, A., Grancomano, D., Dunning, J. B. & Pijanowski, B. C. 2017. Future directions for soundscape ecology: The importance of ornithological contributions. *The Auk,* 134**,** 215-228. 10.1642/AUK-16-124.1

Gasc, A., Sueur, J., Jiguet, F., Devictor, V., Grandcolas, P., Burrow, C., Depraetere, M. & Pavoine, S. 2013. Assessing biodiversity with sound: Do acoustic diversity indices reflect phylogenetic and functional diversities of bird communities? *Ecological Indicators,* 25**,** 279-287.

Gorissen, L., Snoeijs, T., Duyse, E. V. & Eens, M. 2005. Heavy metal pollution affects dawn singing behaviour in a small passerine bird. *Oecologia,* 145**,** 504-509. 10.1007/s00442-005-0091-7

Hallinger, K. K., Zabransky, D. J., Kazmer, K. A. & Cristol, D. A. 2010. Birdsong Differs between Mercury-polluted and Reference Sites. *The Auk,* 127**,** 156-161. 10.1525/auk.2009.09058

Henry, K. A., Cristol, D. A., Varian-Ramos, C. W. & Bradley, E. L. 2014. Oxidative stress in songbirds exposed to dietary methylmercury. *Ecotoxicology,* 24**,** 520-526. https://doi.org/10.1007/s10646-014-1400-x

Hu, Q., Weng, J. & Wang, J. 2010. Sources of anthropogenic radionuclides in the environment: a review. *Journal of Environmental Radioactivity,* 101**,** 426-437. https://doi.org/10.1016/j.jenvrad.2008.08.004

Ishii, Y., Hayashi, S. & Takamura, N. 2017. Radiocesium transfer in forest insect communities after the Fukushima dai-ichi nuclear power plant accident. *PloS One,* 12**,** 1-14.

Jiang, X., Wang, J., Pan, B., Li, D., Wang, Y. & Liu, X. 2022. Assessment of heavy metal accumulation in freshwater fish of Dongting Lake, China: Effects of feeding habits, habitat preferences, and body size. *Journal of Environmental Sciences,* 112**,** 355-365.

Kapustka, L. A. 2004. Issue paper on the ecological effects of metals.: US Environmental Protection Agency.

Kimura, M., Darbro, J. M. & Harrington, L. C. 2010. Avian malaria parasites share congeneric mosquito vectors. *Journal of Parasitology,* 96**,** 141-151.

Klerks, P. L. & Levinton, J. S. 1989. Effects of heavy metals in a polluted aquatic ecosystem. *In:* Levin, S. A., Kelly, J. R., Harwell, M. A. & Kimball, K. D. (eds.) *Ecotoxicology: Problems and Approaches.* New York, NY: Springer.

Koller, L. D. 1980. Immunotoxicology of heavy metals. *International Journal of Immunopharmacology,* 2**,** 269-270.

Krivolutski, D. A., Lebedeva, K. V. & Shuktomova, I. I. 1999. Birds as objects in bioindication of radioactive pollution. *Acta Biologica Hungarica,* 50**,** 145-160. https://doi.org/10.1007/BF03543038

Leaphart, J. C., Korotasz, A. M., Bryan Jr., A. L. & Beasley, J. C. 2020. Environmental fate of radiocesium in biota inhabiting a contaminated ecosystem on the U.S. Department of Energy's Savannah River Site. *Journal of Environmental Radioactivity,* 222. https://doi.org/10.1016/j.jenvrad.2020.106321

Leonzio, C., Bianchi, N., Gustin, M., Sorace, A. & Ancora, S. 2009. Mercury, lead and copper in feathers and excreta of small passerine species in relation to foraging guilds and age of feathers. *Bulletin of Environmental Contamination and Toxicology,* 83**,** 693-697. https://doi.org/10.1007/s00128-009-9789-2

Lin, Y.-P., Anthony, J., Mukhtar, H. & Lin, C.-M. 2021. A spatial prioritization method for identifying potential eco-risk distributions of heavy metals in soil and birds. *Ecotoxicology and Environmental Safety,* 220**,** 112383.

Logue, D. M., Sheppard, J. A., Walton, B., Brinkman, B. E. & Medina, O. J. 2020. An analysis of avian vocal performance at the note and song levels. *Bioacoustics,* 29**,** 709-730. https://doi.org/10.1080/09524622.2019.1674693

Lonvik, K. & Thingstad, P. G. 1999. Radiocaesium accumulation from Chernobyl fallout in nestlings of two pied flycatcher populations (aves) in central Norway; estimating ecological timelag reponses and transfer mechanisms. *Journal of Environmental Radioactivity,* 46**,** 153-169. https://doi.org/10.1016/S0265-931X(98)00122-2

Mireji, P. O., Keating, J., Hassanali, A., Mbogo, C. M., Muturi, M. N., Githure, J. I. & Beier, J. C. 2010. Biological cost of tolerance to heavy metals in the mosquito *Anopheles gambiae*. *Medical and Veterinary Anthropology,* 24, 101-107. 10.1111/j.1365-2915.2010.00863.x.

Morales, G., Vargas, V., Espejo, D., Poblete, V., Tomasevic, J. A., Otondo, F. & Navedo, J. G. 2022. Method for passive acoustic monitoring of bird communities using UMAP and a deep neural network. *Ecological Informatics*. https://doi.org/10.1016/j.ecoinf.2022.101909

Neff, E. & Dharmarajan, G. 2021. The direct and indirect effects of copper on vector-borne disease dynamics. *Environmental Pollution,* 269. https://doi.org/10.1016/j.envpol.2020.116213

Nowicki, S., Peters, S. & Podos, J. 1998. Song learning, early nutrition and sexual selection. *American Zoology,* 38, 179-190.

Parkhurst, C. R. & Thaxton, P. 1973. Toxicity of mercury to young chickens: effect on growth and mortality. *Poultry Science,* 52, 273-276. https://doi.org/10.3382/ps.0520273

Paul, R. E. L., Ariey, F. & Robert, V. 2003. The evolutionary ecology of Plasmodium. *Ecology Letters,* 6, 866-880.

Pijanowski, B. C. 2024. *Principles of Soundscape Ecology,* Chicago, USA, The University of Chicago Press.

Pijanowski, B. C., Villanueva-Rivera, L. J., Dumyahn, S. L., Farina, A., Krause, B. L., Napoletano, B. M., Gage, S. H. & Pieretti, N. 2011. Soundscape Ecology: The Science of Sound in the Landscape. *BioScience,* 61, 203-216.

Roy, A., Queirolo, E., Peregalli, F., Mañay, N., Martínez, G. & Kordas, K. 2015. Association of blood lead levels with urinary F2-8α isoprostane and 8-hydroxy-2-deoxy-guanosine concentrations in first-grade Uruguayan children. *Environmental Research,* 140, 127-135.

Ruhl, L., Vengosh, A., Dwyer, G. S., Hsu-Kim, H., Deonarine, A., Bergin, M. & Kravchenko, J. 2009. Survey of the potential environmental and health impacts in the immediate aftermath of the coal ash spill in Kingston, Tennessee. *Environmental Science and Technology,* 43, 6326-6333. https://doi.org/10.1021/es900714p

Santiago-Alarcon, D., Palinauskas, V. & Schaefer, H. M. 2012. Diptera vectors of avian Haemosporidian parasites: Untangling parasite life cycles and their taxonomy. *Biology Review,* 87, 928-964.

Scheuhammer, A. M. 1987. The chronic toxicity of aluminium, cadmium, mercury, and lead in birds: A review. *Environmental Pollution,* 46, 263-295. https://doi.org/10.1016/0269-7491(87)90173-4

Sievers, M., Hale, R., Parris, K. M. & Swearer, S. E. 2018. Impacts of human-induced environmental change in wetlands on aquatic animals. *Biological Reviews,* 93, 529-554. https://doi.org/10.1111/brv.12358

Silva, A. E., Speakman, R. J., Barns, B. F., Coyle, D. R., Leaphart, J. C., Abernethy, E. F., Turner, K. L., Rhodes Jr., O. E., Beasley, J. C. & Gandhi, K. J. K. 2023. Bioaccumulation of contaminants in Scarabidae and Silphidae beetles at sites polluted by coal combustion residuals and radiocesium. *Science of The Total Environment,* 904, 166821. https://doi.org/10.1016/j.scitotenv.2023.166821

Staicer, C. A., Spector, D. A. & Horn, A. G. 1996. The dawn chorus and other diel patterns in acoustic signaling. *Ecology and evolution of acoustic communication in birds.* Ithaca, NY: Cornell University Press.

Sternalski, A., Matsui, S., Bonzom, J. M., Kasahara, S., Beaugelin-Seiller, K., Ueda, K., Watanabe, M. & Adam-Guillermin, C. 2015. Assessment of radiological dose rates in passerine birds after the Fukushima nuclear accident according to their life stage. *Japanese Journal of Ornithology,* 64, 161-168. https://doi.org/10.3838/jjo.64.161

Sullivan, S. M. P. & Rodewald, A. D. 2012. The energetic pathways that move contaminants from aquatic to terrestrial environments. *Environmental Toxicology and Chemistry,* 31, 1175-1183.

Sulloway, H. M. 2012. Data summary report for Hanford Site coal ash characterization. Washington Closure Hanford.

Sung, H.-C. & Handford, P. 2020. Song characters as reliable indicators of male reproductive quality in the Savannah Sparrow (<i>Passerculus</i> <i>sandwichensis</i>). *Canadian Journal of Zoology,* 98, 32-38. 10.1139/cjz-2019-0018

Towsey, M., Wimmer, J., Williamson, I. & Roe, P. 2014. The use of acoustic indices to determine avian species richness in audio-recordings of the environment. *Ecological Informatics,* 21**,** 110-119.

UNSCEAR 2000. Sources and effects of ionizing radiation, 2000 report. New York: United Nations.

Valkiunas, G. 2004. *Avian Malaria Parasites and Other Haemosporidia,* Boca Raton, FL, USA, CRC Press.

Wada, H. & Coutts, V. 2021. Detrimental or beneficial? Untangling the literature on developmental stress studies in birds. *Journal of Experimental Biology,* 224. 10.1242/jeb.227363

Warren, J. E. & Klaine, S. J. Cadmium and mercury effects on cellular immunity in terrestrial arthropods. Society of Environmental Toxicology and Chemistry, 1995 Pensacola, FL, United States.

Waters, C. N., Syvitski, J. P. M., Galuszka, A., Hancock, G. J., Zalasiewicz, J., Cearreta, A., Grinevald, J., Jeandel, C., McNeill, J. R., Summerhayes, C. & Barnosky, A. 2015. Can nuclear weapons fallout mark the beginning of the Anthropocene Epoch? *Bulletin of the Atomic Scientists,* 71**,** 46-57. https://www.doi.org/10.1177/0096340215581357

CHAPTER 2

UPTAKE OF METALS, METALLOIDS, AND RADIOCESIUM VARIES WITH HABITAT USE AMONG PASSERINE COMMUNITIES AT COAL COMBUSTION AND NUCLEAR FISSION LEGACY WASTE SITES

Werner, C. S., Chapman, M., Rhodes Jr., O. E. & DeVault, T. L. Uptake of metals, metalloids, and radiocesium varies with habitat use among passerine communities at coal combustion and nuclear fission legacy waste sites. Accepted by *Environmental Pollution*. Reprinted here with permission of publisher, 2025.

ABSTRACT

Releases of coal combustion and nuclear fission wastes create contaminated landscapes that pose long-term management challenges. Efforts to facilitate the natural attenuation of legacy wastes in the environment can provide attractive habitat for passerine birds. Passerines have diverse foraging and nesting behaviors that lead to heterogenous contaminant exposure, yet few studies investigate contaminant uptake in passerines on a community scale. This study evaluated whether variation in habitat use strategies among passerines predicted the ongoing uptake of waste-derived elements by birds inhabiting coal combustion and nuclear fission legacy waste areas on the Savannah River Site in South Carolina. Blood concentrations of selenium, arsenic, mercury, zinc, copper, and lead were measured in 362 birds from 35 species. Whole-body radioactivity concentrations due to cesium-137 were measured *in vivo* in 143 birds from 31 species using a novel, field-based gamma spectrometry system. Generalized linear mixed effects models were used to evaluate whether trophic category and degree of terrestriality predicted contaminant burdens among passerine communities. Selenium, mercury, arsenic, and cesium-137 were elevated in passerines inhabiting legacy waste sites compared to those at reference sites. Blood concentrations of selenium and mercury varied by trophic category, whereas arsenic and cesium-137 increased with degree of terrestriality. The behavioral correlates of contaminant uptake among passerines provide insight into the mobility of waste-derived elements in ecosystems and inform species-level risk assessments. Future studies should use *in vivo* gamma spectrometry to conduct long-term field studies that evaluate the effects of internal radiation in small-bodied wildlife.

INTRODUCTION

Legacy wastes from energy and defense production pose an enduring threat to environmental health (Dwivedi et al., 2022). Coal combustion and nuclear fission generate byproducts that, when disposed of improperly, serve as sources of potentially toxic elements to ecosystems (Dwivedi et al., 2022, Deonarine et al., 2023, Hu et al., 2010). Coal-fired power stations store billions of tons of coal combustion residuals (CCR) in aging impoundments, the failure of which causes an influx of concentrated metals and metalloids to surrounding environments (Deonarine et al., 2023). The nuclear industry has spurred the unintentional release of nuclear fission products (NFP), including long-lived radionuclides, through atmospheric fallout and local releases of contaminated effluent (Hu et al., 2010). The metals, metalloids, and radionuclides derived from CCR and NFP can interfere with the basic physiological processes of living organisms and pose a significant health risk upon their entry into the environment (Kapustka, 2004, Sample and Irvine, 2011). Their toxicity and persistence create long-term challenges for the management of contaminated lands globally.

In the United States, the U.S. Environmental Protection Agency (US EPA) serves as the primary regulatory authority regarding risk assessment and remediation requirements on sites contaminated with CCR and NFP. Due to the high costs associated with excavating legacy wastes, the US EPA, state regulators, and responsible parties often employ a variety of *in situ* remediation techniques that aim to immobilize and attenuate waste-derived elements in the environment, along with administrative controls that restrict land use (Carlon et al., 2009). Consequently, a growing acreage of land in the U.S. is deemed unfit for residential, agricultural, or recreational purposes due to the presence of contaminants that create persistent threats to human health (USEPA, 2024).

Although land-use restrictions reduce human exposure to residual contamination, they often fail to exclude wildlife. Infrastructure built to sequester contaminants, including constructed wetlands or landfills, may even attract wildlife to legacy waste sites (Sievers et al., 2018, Seewagen and Newhouse, 2018). These landscape features, along with the limited human activity on contaminated lands, can create refuges for wildlife driven from surrounding areas by rapid habitat loss (Coates, 2013, Schlichting et al., 2020). However, the colonization of legacy waste sites by wildlife can lead to the integration of waste-derived elements into dynamic ecological processes that enhance their bioavailability and alter their environmental fates (Brandt et al., 2019, Avery, 1996, Klerks and Levinton, 1989, Silva et al., 2023). Additionally, contaminant uptake can impact wildlife health and reduce population viability, causing waste sites to serve as ecological traps rather than valuable refuges (Sievers et al., 2018, Hale and Swearer, 2016, Thomas et al., 2021). The proper management of legacy waste in the environment therefore requires an understanding of the uptake of waste-derived elements by wildlife attracted to remediation sites.

Passerine and near-passerine birds (hereafter "passerines") are small-bodied, flighted, and adapted to diverse environments, allowing them to colonize legacy waste sites with relative ease. Consequently, passerines are often observed in constructed wetlands, capped landfills, abandoned chimneys, and other structures characteristic of remedial sites that provide nesting and foraging habitat in the absence of natural wetlands, grasslands, and old growth trees (Sievers et al., 2018, Seewagen and Newhouse, 2018, Laughlin et al., 2022). Within such habitats, the threats that various waste-derived trace metals, metalloids, and radionuclides pose to passerines are difficult to characterize given the complex, interconnected processes in which these elements circulate (Werner et al., 2024, Tsipoura et al., 2008). The absorption rates of trace elements depend on their chemical speciation and can vary widely across trophic levels, feeding guilds, and habitat preferences of different bird species (Scheuhammer, 1987). Some contaminants bioaccumulate in the tissues of prey organisms (Abbasi et al., 2015, Leonzio et al., 2009) or biomagnify up food chains (Ackerman et al., 2016), increasing rates of exposure for birds at higher trophic levels. Others bind readily to sediments and may accumulate in birds that incidentally ingest soil while foraging or building nests (Avery, 1996, Kennamer et al., 1998). Exposure pathways can differ for co-occurring contaminants, and a thorough assessment of contaminant uptake by passerines at legacy waste sites benefits from the inclusion of multiple species that span a variety of habitat use strategies.

Studies that investigate the transfer of contaminants to passerines inhabiting CCR or NFP waste sites often focus on very few, or even a single, indicator species chosen to represent the broader taxonomic group (Cooper et al., 2017, Bryan et al., 2003, Beck et al., 2014, Lonvik and Thingstad, 1999). A single-species approach limits the natural variation found in field settings and is useful for maximizing the power of monitoring initiatives that aim to detect waste-derived contaminants in bioindicators, which is particularly important in studies that rely on lethal sampling. Examples include the use of common grackle eggs to investigate trace metal transfer from active CCR storage basins in South Carolina, USA (Bryan et al., 2003), and the use of tree swallow nestlings along the Emory and Clinch River following the 2008 TVA Kingston Fossil Plant CCR spill (Beck et al., 2014, Walls et al., 2014). In both cases, selenium (Se) was the only element consistently transferred to each bird species (Bryan et al., 2003, Walls et al., 2014). However, a reliance on single-species indicators has led to a paucity of data on trace element concentrations for most passerine species inhabiting CCR-affected lands, and thus variation in the uptake of Se or other metals within passerine communities remains unknown.

The transfer of NFP-derived radionuclides to passerines has primarily been investigated within the Chernobyl and Fukushima exclusion zones or in remote locations affected by fallout from these accidents (Beresford et al., 2016, Baeza et al., 1991, Lonvik and Thingstad, 1999, Krivolutski et al., 1999, Sternalski et al., 2015). These studies suggest that variation in radionuclide uptake occurs between passerine species, but their small sample sizes limit conclusions on the dietary or behavioral correlates of uptake. Aside from the major accidents at Chernobyl and Fukushima, local-scale releases of NFP contaminate reactor cooling ponds, canals, and outflow streams that provide attractive habitat for passerines (Hu et al., 2010, Dwivedi et al., 2022). The potential for these aquatic systems to serve as sources of radionuclides for terrestrial birds is not known. Passerines can transport radiological material from localized contamination zones, and therefore a greater understanding of radionuclide uptake in the passerines species attracted to these areas is critical for proper radiological protection (Baeza et al., 1991, Chapman et al., 2022).

The persistence of CCR and NFP in the environment and the abundance of passerines in legacy waste areas warrant further study of contaminant transfer among broader passerine communities. Accordingly, we have two goals for conducting this study. First, we seek to investigate the ongoing uptake of waste-derived trace elements by passerines at sites where *in situ* remediation of CCR and NFP has occurred. To address this first goal, we hypothesize that (1) passerines inhabiting CCR sites will exhibit higher concentrations of six waste-derived elements—zinc (Zn), copper (Cu), selenium (Se), lead (Pb), arsenic (As), and mercury (Hg)—in their blood than will those at reference sites, and (2) passerines inhabiting NFP sites will exhibit higher radioactivity due to an abundant and long-lived fission byproduct, cesium-137 (^{137}Cs), compared to passerines at reference sites. Second, we seek to identify whether differences in habitat use—specifically dietary category and foraging and nesting behavior— predict variation in trace element and radionuclide uptake. To address this second goal, we hypothesize that (1) ground-foraging and ground-nesting birds are more likely to disturb soils contaminated with trace elements and radionuclides, resulting in greater opportunity for contaminant uptake as compared to species that primarily nest and forage in the forest understory and canopy, and (2) that carnivorous birds are more likely to accumulate contaminants due to their higher trophic position as compared to birds that are primarily herbivorous or omnivorous. This study will fill gaps in our knowledge of baseline trace element concentrations in field populations, help refine the roles of biotic and abiotic factors in contaminant transfer, and identify passerine guilds for prioritized research and conservation in contaminated habitat.

METHODS

Site Description

The Savannah River Site (SRS) is owned and operated by the U.S. Department of Energy (US DOE) and encompasses a 780 km^2 property on the upper coastal plain of South Carolina. At the height of production in the 1950s and 1960s, nine coal-fired power plants provided electricity to the site and five nuclear reactors manufactured tritium and plutonium for the U.S. nuclear weapons arsenal (Palmer, 1997, Carlton et al., 1992, White and Gaines, 2000). Coal combustion residuals were stored in unlined surface impoundments that were subject to leaching and spillover until the closure of the last remaining coal-fired power plant in 2012 (Palmer, 1997). Several thousand GBqs of NFP were released to SRS reservoirs and streams from leaking nuclear reactor components, including approximately 7.0×10^{13} Bq of ^{137}Cs (Carlton et al., 1992). Cesium-137 shows high affinity for the clay sediments of the region, and an estimated 65% of the released ^{137}Cs has remained on SRS property within cooling ponds, canals, and receiving streams (Carlton et al., 1992).

This study occurred at six sites on the SRS that contain riparian or wetland habitat used by a variety of breeding passerines (Figure 2.1). These habitats surround freshwater systems that are either natural (e.g. stream, Carolina bay) or constructed (e.g. cooling water canal, stormwater retention pond) and are under varying remediation plans outlined by the US DOE, US EPA, and South Carolina Department of Environmental Services.

Two of the six sites serve as uncontaminated reference (Ref) habitat. Craig's Pond (Ref 1) and Upper Three Runs (Ref 2) are research set-aside areas that were recommended for preservation by the U.S. Atomic Energy Commission in 1952 and have no known history of point-source contamination from SRS activities (White and Gaines, 2000). D Area (CCR 1) contains an overflow CCR plume, one coal pile runoff basin, and several CCR basins. In 2016, a portion of the CCR at D-Area was consolidated to a single capped landfill, and the remainder was subject to natural attenuation (SRNS, 2020). Passerine sampling occurred around a stormwater retention pond and along Beaver Dam Creek, both of which receive runoff from D Area. H Area (CCR 2) contains a coal pile runoff basin, a CCR basin, and an overflow CCR plume. The H Area basins have not been excavated or capped and are frequently flooded, and the primary management plan involves natural attenuation (SRNS, 2020). Passerine sampling occurred along McQueen's Branch, a stream that receives runoff from H Area.

R Area (NFP 1) operated the R Reactor beginning in 1953. NFP-contaminated cooling water flowed from R Reactor through a series of pre-cooler ponds and canals before emptying into a reservoir and then into Lower Three Runs Creek. Passerine sampling occurred along the first pre-cooler pond and canal. L Area (NFP 2) contains L Lake, a cooling reservoir that received effluent from two reactors and that flows into Steel Creek. Passerine sampling occurred below the lower junction of Steel Creek and the cooling reservoir.

Trace Element Measurement

We sampled passerine communities for trace metals and metalloids at CCR and reference sites from March to July of 2023. We spent two weeks at each site before rotating, and we visited all sites twice. On each day of sampling, we opened four to ten 30 mm mist nets within 100 meters of contaminated or reference freshwater systems. Nets were opened from sunrise to approximately 1100 and checked every 20 minutes. Song callbacks attract birds to mist nets (Johnson et al., 1981, Nesbitt et al., 1982) and were used in this study to increase the rate of capture and to ensure even sampling of birds with varying habitat use strategies across sites. The same mist-net locations were used for replicate site visits, and the daily number and location of opened nets, as well as the use of song callbacks, were adjusted throughout the field season based on fluctuating capture rates (Marques et al., 2013) and microhabitat preferences of present species.

We recorded the species and body mass of each bird and attached an aluminum USGS leg band at the time of capture. We then collected 0.07-0.30 mL of blood from the jugular vein using an insulin syringe fitted with a 28-gauge needle, ensuring the sample did not exceed 1% of the bird's body weight. Whole blood samples were stored in metal-free vials and transferred to a 0 °C freezer in the laboratory on the same day.

In the laboratory, blood samples were freeze-dried, powdered, and homogenized by stirring. Dried samples with masses of 10.0 -18.0 mg or 18.1-50.0 mg were digested in 0.54 mL or 1.00 mL solutions containing equal parts nitric acid and hydrogen peroxide on a hot block (Corning LSE Digital Dry Bath) before they were diluted with molecular-grade water to reach a 6% acid concentration. Samples were then analyzed for Zn, Cu, Pb, As, and Se using an Inductively-Coupled Plasma Mass Spectrometer NexION 300X (PerkinElmer). For a subset of samples with larger blood volume, 5 mg of dried blood was separated and analyzed for Hg in a Direct Mercury Analyzer (DMA-80, Milestone, Shelton, CT, USA). Reference materials of known metal concentrations (TORT-3 and DOLT-5), and a blank were included with each set of samples. No duplicate samples were run due to the small amount of blood available. Method detection limits (MDL) were calculated for each metal based on sample volume and instrument sensitivity. All concentrations are reported on a dry weight basis.

Cesium-137 Measurement

We measured the activity concentrations of ^{137}Cs in passerines at NFP and reference sites from February to April 2023 using a portable high-purity germanium (HPGe) Aegis-BE5030 Spectrometer (BE5030 crystal, Mirion Technologies) and in June 2023 using a second HPGe Aegis-GC40 Spectrometer (40% GC crystal, Mirion Technologies). The second spectrometer was used after the first required servicing. We spent a total of two weeks at each site. Energy calibration was conducted for each Aegis in advance to establish a relationship between the ^{137}Cs decay energy (662 keV) and the corresponding channel in the Genie™ 2000 Spectroscopy Software (Mirion Technologies). Prior to starting fieldwork each morning, we counted a ^{137}Cs chip source to ensure that the device remained calibrated, and we adjusted the gain parameter of the Aegis to correct the relationship if necessary.

In the field, the Aegis was attached to a 27.9 cm by 40.6 cm deep front opening, split-top lead shield with 10 cm wall thickness mounted within a trailer. To determine the minimum counting time required for our system, we first constructed a custom sample geometry representing one standard bird using Laboratory Sourceless Calibration Software (LabSOCS™ S573, Mirion Technologies). Differences in gamma attenuation within living tissue and water can be negligible (Rafiei et al., 2020). Therefore, we modeled a standard bird as water within a polyethylene cylinder with dimensions from those of the average Northern cardinal (*Cardinals cardinalis*). Separate geometries were constructed for Aegis-BE5030 (end-cap diameter: 10.16 cm; end-cap length 10.10 cm) and Aegis-GC40 (end-cap diameter: 8.255 cm; end-cap length: 11.10 cm) using their respective characterization files, and the counting efficiency of ^{137}Cs for each modeled geometry was determined by LabSOCS™. We calculated the expected minimum detectable activity (MDA) of ^{137}Cs according to Currie (1968) and determined that a counting time of 30 minutes was sufficient to achieve a counting uncertainty of <10%.

Passerines were captured in mist-nets and fitted with aluminum USGS bands as described above. Each bird was secured in a cotton bag within a polyethylene cylinder and placed on an acrylic platform in the shielded chamber for a 30-minute count time. One field blank was counted each day. We analyzed spectra with Genie™ 2000 following analysis sequence parameters recommended for HPGe detectors (Mirion Technologies). The presence of a ^{137}Cs peak was accepted if the observed net peak area remained above the Critical Level (Lc; $\alpha = 0.05$), calculated according to Currie (1968), after applying a standard background subtraction derived from the field blank sample spectra collected on the corresponding day. Net peak areas were then corrected using an interpolated efficiency function tailored for the geometry of our counting system before the radioactivity (in Bq) was calculated in the ^{137}Cs region of interest.

Data Analysis

We used an information theoretic approach to evaluate whether site contaminant history, terrestriality, and trophic category predicted blood trace metal concentrations or ^{137}Cs activity concentrations in passerines. To approximate relative time spent on the ground for different species, we assigned a "ground score" based on average nesting and foraging heights reported by Ehrlich et al. (1988). Species that both nest and forage on the ground were assigned a ground score of two, while species that either nest or forage on the ground were assigned a score of one. All other species received a ground score of zero. We approximated the dominant trophic levels of passerine species by assigning each as an "herbivore", "omnivore", or "carnivore" according to the categories used by the AVONET database (Pigot et al., 2020, Tobias et al., 2022).

We evaluated a set of candidate generalized linear mixed effects models (GLMMs) to determine whether the categorical variables (1) site contaminant history (CCR, NFP, or Ref), (2) ground score (0, 1, or 2), and (3) trophic category predicted blood concentrations (ppm *dw*) of Zn, Cu, Se, Hg, As, and Pb, and activity concentrations of ^{137}Cs. We included species as a random effect. The distributions of trace element concentrations in the blood ranged from log normal to heavily right-skewed. Therefore, we evaluated both gaussian and gamma distributions with log links and chose the most appropriate distribution for each response variable by examining residuals with the DHARMa package (Bolker, 2008, Hartig, 2022). Cesium-137 activities and trace element concentrations with a majority of values <MDL were converted to binomial variables indicating whether or not they were detected in a sample and were analyzed using a binomial distribution and logit link.

We chose the best fitting GLMM by comparing the AIC of candidate models using the *lme4* package (Bates et al., 2015). We began model selection with the null model and systematically evaluated 13 candidate models containing all combinations of the three predictors, as well as interactions involving site contaminant history. We considered a variable or an interaction to be predictive of trace element concentrations or ^{137}Cs activities if it was included in the model with the lowest AIC, and we report coefficients from the top model for each response variable after applying the relevant inverse link function. Additionally, we evaluated the strength of evidence for the significance of each predictor by examining its presence in all candidate models with δAIC < 2. To investigate species-level variability in our response variables, we extracted the conditional means of the random effects and their standard deviations from each top model using the *ranef* function. We report conditional means with 95% confidence intervals above zero, indicating that trace element concentrations or ^{137}Cs activities for a species were elevated above levels predicted by the fixed effects in the top models.

RESULTS

Trace Elements

We captured 362 birds from 35 species for trace metal sampling across CCR and reference sites. Most individuals were categorized as carnivores (n = 262), followed by omnivores (n = 63) and herbivores (n = 37). Sixty-one individuals were from species known to nest and forage on the ground (ground score = 2), 95 were from species that either nest or forage on the ground (ground score = 1), and 206 were categorized as spending little time on the ground (ground score = 0; Table 2.1).

Blood concentrations of Zn, Cu, Se, As, and Pb were measured in all captured birds, and blood Hg was measured in a subset of 180 birds. Zinc, Cu, and Hg were detected in all samples. Selenium was detected in 99.1% of samples (MDL = 0.961 ppm *dw*), Pb in 19.3% of samples (MDL = 0.021 ppm *dw*), and As in 11.9% of samples (MDL = 0.039 ppm *dw*). Average percent recoveries for the certified reference materials were within 80% to 120% for all elements (Table A.1).

Generalized linear mixed effects models indicated that site contaminant history and trophic category predicted concentrations of Se in passerine blood (Table 2.2). At reference sites, mean blood Se concentrations were 2.82 ppm *dw* (SE 1.18) in herbivores, 4.20 ppm *dw* (SE 1.42) in omnivores, and 4.24 ppm *dw* (SE 1.39) in carnivores (Figure 2). Blood Se was predicted to increase above reference site values by 31% (SE 4%) in birds at CCR sites (Table 2.2; Table A.2). Site contaminant history was included as a predictor of blood Se in all four candidate models.

Site contaminant history, trophic category, and an interaction between these terms predicted blood concentrations of Hg in the top GLMM. We observed wider variation in blood Hg compared to other elements (Figure 2.2). At reference sites, mean blood Hg was 0.23 ppm *dw* (SE 1.89 ppm) for herbivores, 0.37 ppm *dw* (SE 3.95) for omnivores, and 0.49 ppm *dw* (SE 3.73) for carnivores. The effect of site contaminant history on blood Hg was modified by trophic category. Blood Hg increased by 47% (SE 19%) in herbivorous birds at CCR sites compared to reference sites, but did not increase in carnivorous or omnivorous birds (Table 2.2; Figure 2.2). The null model competed closely with the top GLMM for Hg ($\delta = 0.78$).

Birds inhabiting CCR sites and those that forage and nest on the ground were more likely to have detectable As in their blood (Table 2.2). The top GLMM for As included ground score, site contaminant history, and an interaction between these terms but was followed closely by a competing model that contained ground score as the sole predictor of As in the blood ($\delta = 0.36$).

Blood concentrations of Zn, Cu, and Pb were not elevated at CCR sites compared to reference sites. However, birds that nest and/or forage on the ground had higher blood concentrations of Zn (β 1.08, SE 1.04) and were more likely to have detectable blood Pb (β 0.74, SE 0.60) than those that nest and/or forage in the understory and canopy.

The random effect for species explained additional variability in blood trace element concentrations (Table 2). The conditional means of the random effects (*u*) indicated that observed blood trace element concentrations were higher for some species than was predicted by the fixed effects alone (Figure 2.4). Among the most abundant species ($n \geq 3$ per site type), species-level deviance from predicted blood concentrations was highest for Hg in Louisiana waterthrushes (*Parkesia motacilla*; *u* 3.11, SE 1.08), and Carolina wrens (*Thryothorus ludovicianus*; *u* 2.66, SE 1.02), followed by Se in *P. motacilla* (*u* 1.94, SE 1.01), red-eyed vireos (*Vireo olivaceus*; *u* 1.29, SE 1.02), summer tanagers (*Piranga rubra*; *u* 1.20, SE 1.02), great-crested flycatchers (*Myarchus crinitus*; *u* 1.17, SE 1.02), and white-eyed vireos (*Vireo griseus*; *u* 1.06, SE 1.00). The likelihood of detecting As in the blood was higher than expected for *P. rubra* (*u* 0.83, SE 0.53), Carolina chickadees (*Poecile carolinensus*; *u* 0.61, SE 0.54), and *C. cardinalis* (*u* 0.57, SE 0.52).

Cesium-137

We measured ^{137}Cs activity in 143 birds of 31 species at NFP and reference sites, including 98 individuals categorized as carnivores, 28 as omnivores, and 17 as herbivores. Sixty-nine individuals had a ground score of 2, 44 had a ground score of 1, and 30 had a ground score of 0.

The portable HPGe counting system had an average minimum detectable activity of 1.75 (SD 1.07) Bq. We detected ^{137}Cs activity in 27 birds with activity levels ranging from 0.004 Bq/g *ww* to 0.16 Bq/g *ww*, with a mean of 0.05 Bq/g *ww* (SD 0.04). Cesium-137 was more likely to be detected in birds inhabiting NFP sites and in those that forage and nest on the ground (Table 2.2; Figure 2.3). Site contaminant history was included in all three candidate models, whereas "ground" was included in the top two models. Detector was also present in all candidate models, and Aegis-BE5030 was significantly more likely to detect ^{137}Cs than Aegis-GC40. The conditional means of the random effects indicated that the likelihood of detecting ^{137}Cs was higher than expected for common yellowthroats (*Geothylpis trichas*; *u* 0.70, SE 0.52) and *C. cardinalis* (*u* 0.63, SE 0.54; Figure 2.4).

DISCUSSION

The transfer of waste-derived metals, metalloids, and radionuclides to biotic compartments of an ecosystem depends on multiple factors, including their chemical speciation and physiological roles, the pH and redox state of the environment, and the ecological dynamics that lead to chemical exchange between organisms (Brandt et al., 2019, Fernández-Martínez and Charlet, 2009). Elevated trace elements in passerine birds inhabiting legacy waste sites indicate the alignment of multiple processes that foster element mobility and bioavailability. We sampled the passerine community in wetland and riparian habitats designated for long-term, *in situ* remediation of coal combustion and nuclear fission wastes to evaluate the transfer of waste-derived contaminants to highly mobile, terrestrial wildlife. We found that Se was the primary contaminant of concern in passerines at CCR sites, whereas ^{137}Cs activity was elevated in passerines at NFP sites.

Selenium is an essential micronutrient sourced primarily from the diets of organisms, and concentrations in the blood reflect recent dietary uptake (Puls, 1988, Fernández-Martínez and Charlet, 2009). The trophic transfer of Se to passerines is not well characterized due to their highly variable diets and the wide range of Se concentrations in their potential prey items. (Fletcher et al., 2017, Silva et al., 2023, Presser and Luoma, 2010). We found that reference blood Se concentrations varied by a factor of three among species, with the lowest concentrations observed in Northern cardinals (*Cardinalis cardinalis*; $\bar{x}$ 2.61 ppm *dw* SD 1.31, n=11) and the highest in Louisiana waterthushes (*Parkesia motacilla*; $\bar{x}$ 7.95 ppm *dw* SD 3.68, n=5). Blood Se was significantly lower in birds categorized as herbivorous compared to those categorized as omnivorous or carnivorous by the AVONET database, indicating that these broad groupings capture variation in the Se content of breeding season diets for passerines at our study sites. The use of CCR habitat was associated with a 31% increase in blood Se regardless of whether birds were primarily herbivorous, omnivorous, or carnivorous. Widespread Se enrichment across trophic categories suggests that CCR-derived Se enters the base of the food web at remedial sites and accumulates in multiple plant and animal species that contribute to the varied diets of passerines.

The passerines sampled at CCR sites in this study forage and/or nest near constructed wetlands and streams that receive stormwater effluent. The exposure of CCR to rainwater allows soluble chemical species, including selenate, to leach into runoff (Gerson et al., 2022). Wetlands foster microbial communities that transform selenate to its more bioavailable chemical species, selenite, which is readily taken up by hyperaccumulator plants, algae, and aquatic macroinvertebrates and transferred through diverse food web pathways (Hopkins et al., 2005, El Mehdawi and Pilon-Smits, 2011, Fletcher et al., 2014). The continued transformation of selenate to selenite and to organo-Se can lead to a steady build-up of Se in maturing wetland environments (Presser and Luoma, 2010). The present study, along with prior research conducted at the same CCR sites, suggests that Se is consistently transferred to a variety of wildlife despite efforts to contain and remediate CCR (Cooper et al., 2017, Oldenkamp et al., 2017, Haskins et al., 2017). Prior to the cessation of coal combustion and the consolidation of CCR into a capped landfill, Bryan et al. (2003) found that the eggs of common grackles (*Quiscalus quiscula*) nesting near D-Area CCR basins had significantly elevated Se ($\bar{x}$ 5.88 ppm *dw*, SD 0.44, n = 14) compared to those at reference sites ($\bar{x}$ 2.69 ppm *dw*, SD 0.13, n = 12). Two decades later, we observed comparable Se levels in the blood of *Q. quiscula* nesting at D-Area ($\bar{x}$ 6.92 ppm *dw*, SD 1.04, n = 8) and at reference sites ($\bar{x}$ 3.60 ppm *dw*, SD 0.30, n = 2). Other recent studies conducted on the SRS have observed elevated

Se in species that forage in and around aquatic systems receiving CCR runoff, including waterfowl (Oldenkamp et al., 2017), aquatic turtles (Haskins et al., 2017), and small fossorial mammals, herpetofauna, and invertebrates (Holland 2024) . Taken collectively, these findings suggest that CCR-derived Se does not naturally attenuate in maturing wetland environments but remains highly mobile and bioavailable decades after waste input.

The concentrations that define Se deficiency, adequacy, and toxicity in passerines are not well known, especially in the presence of As, Hg, and other elements that may interact with Se (Gerson et al., 2020). Excess Se in the blood of breeding birds can transfer to eggs and reduce clutch viability, and a mean blood Se concentration of 4.8 ppm *dw* has been suggested as a level of concern warranting further study on reproductive impairment in birds (Eisler, 2000, Ohlendorf and Heinz, 2011). Mean blood Se concentrations at our CCR sites exceeded the level of concern in two thirds of the surveyed species, with the highest values occurring in *P. motacilla* ($\bar{x}$ 11.22 ppm *dw*, SD 3.96, n = 6). However, the percentage increase in blood Se over reference site levels was relatively modest. Evidence of Se toxicity in field populations is mostly derived from aquatic, non-passerine birds inhabiting the Kesterson Reservoir in California, for which Se concentrations were ten times those in nearby reference areas (Ohlendorf et al., 1988). By contrast, no evidence of selenosis was observed in tree swallows at sites affected by a CCR spill in Kingston, Tennessee, where average whole-body Se concentrations were twice those in birds from unaffected habitat (Beck et al., 2014). Arsenic was also elevated in birds captured at CCR our sites, though the maximum detected blood concentration (0.15 ppm *dw*) was below levels at which As toxicity has been observed in passerines (Albert et al., 2009, Sánchez-Virosta et al., 2015). Experimental studies in birds have demonstrated that As can counteract selenosis by lowering the retention time and toxicity of Se, thereby reversing the negative effects of Se on hatching success (Stanley et al., 1994). The ecological impact of low-dose, CCR-derived Se and As on the health and viability of passerine populations is an important direction for future study, especially if CCR basins serve as long-term sources of these metalloids to passerine communities.

We observed a steady increase in average blood Hg concentrations from herbivores to omnivores to carnivores at reference sites. This pattern is consistent with the biomagnification of Hg in organisms at higher trophic levels, a phenomenon that is well-documented in birds and occurs in freshwater systems globally (Ackerman et al., 2016). Sediment samples from our study sites indicate that CCR serve as a source of inorganic Hg to SRS environments (Fletcher et al., 2019, SRNS, 2020). We therefore expected that passerines would accumulate more Hg at CCR sites compared to reference sites, with the highest accumulation occurring in carnivores. Although mean blood Hg varied by a factor of ten across species at CCR sites, concentrations did not consistently increase and instead appeared to converge between the trophic categories, with mean observed concentrations increasing in herbivores and decreasing in omnivores and carnivores relative to reference sites. A similar pattern occurred at both the Dan River CCR spill in North Carolina, and the Kingston Fossil Plant CCR spill in Tennessee, where Hg concentrations were elevated in sediments but no evidence of Hg biomagnification was observed (Deonarine et al., 2023, Meyer et al., 2014). Mercury biomagnification primarily depends on two conditions: the influx of inorganic Hg to the environment and the conversion of inorganic Hg to its bioavailable form, methylmercury (MeHg; Evers et al., 2007). The lack of evidence for biomagnification observed here suggests that the methylation of CCR-derived Hg may be limited at our sites. Further investigation is required to evaluate factors that may inhibit the trophic transfer of Hg derived from CCR, including potential interactions with Se (Gerson et al., 2020) and other co-occurring elements.

Long-term disposal of NFP remains a major challenge in the nuclear industry. The ^{137}Cs contamination released to SRS aquatic systems in the 1960s was largely left in place due to its strong affinity for sediment, which limits its mobility (Carlton et al., 1992). We found that passerines inhabiting NFP sites and those that forage and/or nest on the ground were more likely to have detectable, whole-body ^{137}Cs activity, indicating that waste-derived ^{137}Cs is transferred from abiotic compartments to terrestrial birds and that contact with contaminated sediment is the most likely transfer pathway. Due to the non-lethal approach used in this study, we cannot determine whether the ^{137}Cs observed in live birds was passing through the gastrointestinal tract, adhered to feathers, or incorporated into internal tissues. Prior studies have reported elevated ^{137}Cs in the muscle tissue of ground-foraging wildlife (Kennamer et al., 2017, Oldenkamp et al., 2017) and have suggested that incidental ingestion of contaminated sediment is the primary uptake pathway leading to ^{137}Cs bioaccumulation (Beresford et al., 2016, Leaphart et al., 2019). Despite the evidence of ^{137}Cs transfer to passerines at NFP sites, the activity levels observed here are below SRS thresholds of concern and are an order of magnitude lower than historical levels reported in most wildlife on the SRS (Carlton et al., 1992) and in passerines from the Fukushima and Chernobyl exclusion zones (Sternalski et al., 2015, Beresford et al., 2016). Sediments containing elevated ^{137}Cs are mostly submerged in canals, ponds, and streams on the SRS, and the area of contaminated habitat available for ground-foraging and ground-nesting passerines is small relative to their home ranges. Low residence times in contaminated habitat, along with a short biological half-life of ^{137}Cs in passerines and the ongoing decay of ^{137}Cs in the environment, likely limit ^{137}Cs accumulation in passerines on the SRS (Brisbin Jr., 1991). However, fluctuations in water levels at both of our study sites have historically exposed large areas of contaminated sediment, leading to increased ^{137}Cs activity in ground-foraging wildlife (Kennamer et al., 1998). Long-term management of NFP-contaminated cooling ponds should consider the risk of ^{137}Cs uptake by ground-foraging and ground-nesting passerines and minimize the accessibility of radioactive sediments.

To our knowledge, this is the first study to use an HPGe spectrophotometer to conduct *in vivo* measurements of radioactivity concentrations in wildlife. The high resolution of the HPGe spectrometer, along with robust shielding, allowed us to achieve a lower MDA in less counting time compared to previous studies that used sodium iodide scintillation detectors (Bondarkov et al., 2011, Kennamer et al., 1993). This system is therefore suitable for *in vivo* radioactivity quantification in small-bodied wildlife that require shorter handling times. In the absence of portable, field-based counting systems with adequate sensitivity, prior efforts to quantify activity concentrations in wildlife have relied on lethal sampling or opportunistic collection of carcasses (Beaugelin-Seiller et al., 2020). Consequently, data on internal or cutaneous dose rates are unavailable for most wildlife, despite evidence that they contribute more to total radiation exposure than environmental dose rates in vertebrates (Beaugelin-Seiller et al., 2020). The methods presented here provide the opportunity to repeatedly sample activity concentrations in small-bodied animals throughout their lives while simultaneously collecting data on the movements, health, reproduction, and survival of individuals and the stability of affected populations. This represents a major step towards more accurate assessments of radiation doses to wildlife and the potential sublethal effects of chronic, internal or cutaneous exposure in field settings. The probability of ^{137}Cs detection differed significantly between the two Aegis spectrometers used in this study, which can be attributed to the different relative efficiencies of the BE5030 and GC40 HPGe crystals (Mirion Technologies). When counting times are constrained by ethical requirements, detection limits can be reduced by maximizing the efficiency of the HPGe semiconductor.

This study investigated whether two functional traits—dietary category and terrestriality—covary with contaminant burdens among passerine communities inhabiting CCR and NFP legacy waste sites. However, the random effects variance indicated that some species possess additional traits that increase their risk of contaminant uptake. *Parkesia motacilla* are unique among the passerine community in that they nest and forage almost exclusively within the banks of streams and have a dietary reliance on aquatic prey (Mattsson and Cooper, 2009, Mattsson et al., 2020). *Parkesia motacilla* had the highest species-level deviance in blood concentrations of both Hg and Se, and their average blood levels of both contaminants exceeded thresholds associated with reduced egg hatchability (Mattsson et al., 2020, Ackerman et al., 2016, Ohlendorf and Heinz, 2011). Due to their dependence on streams, *P. motacilla* are considered an at-risk species in aquatic systems degraded by agricultural operations (Mattsson and Cooper, 2006), hydraulic fracturing (Latta et al., 2015), and coal mining waste (USEPA, 2011). Another riparian species, *G. trichas*, had the highest species-level deviance in the likelihood of whole-body ^{137}Cs. *Geothylpis trichas* nest near or on the ground in low vegetation that grows on the margins of water, where they establish territories that range from 0.1 to 2.2 ha (Guzy and Ritchison, 2020, Stewart, 1953). Because of their habitat requirements, *G. trichas* at NFP sites are more confined to the periphery of cooling ponds, canals, and streams where the highest concentrations of adsorbed ^{137}Cs occur. In general, riparian passerines inhabiting legacy waste areas have higher fidelity to contaminated aquatic systems than passerines with more flexible habitat requirements, leading to a greater risk of contaminant uptake. Consequently, aquatic systems affected by CCR and NFP may serve as population sinks for riparian birds, thereby compounding the declines that this vulnerable group faces due to the loss of high-quality breeding habitat (Rosenberg et al., 2019). The rate of contaminant transfer from aquatic systems to riparian passerines necessitates long-term studies on reproductive consequences and, potentially, the implementation of management strategies that deter riparian birds from breeding in legacy waste areas.

CONCLUSION

Legacy waste sites provide foraging and nesting opportunities for passerines but also serve as sources of potentially hazardous elements. Selenium is the primary contaminant transferred to passerine communities in habitat affected by CCR. Blood Se concentrations in passerines vary widely with their diets, but Se uptake occurs across dietary types in CCR habitat. Mercury and arsenic are secondary contaminants of concern in CCR habitat, and their uptake is mediated by diet and terrestriality, respectively. Cesium-137 is transferred to passerines from NFP-contaminated cooling ponds and streams, and birds that contact contaminated sediments while foraging and nesting are at higher risk of ^{137}Cs uptake. Future studies should evaluate the effects of low-level Se and ^{137}Cs in passerines and other terrestrial wildlife to further assess the suitability of CCR and NFP legacy waste sites as habitat.

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LITERATURE CITED

Abbasi, N. A., Jaspers, V. L. B., Chaudhry, M. J. I., Ali, S. & Malik, R. N. 2015. Influence of taxa, trophic level, and location on bioaccumulation of toxic metals in bird's feathers: a preliminary biomonitoring study using multiple bird species from Pakistan. *Chemosphere,* 120**,** 527-537. https://doi.org/10.1016/j.chemosphere.2014.08.054

Ackerman, J. T., Eagles-Smith, C. A., Herzog, M. P., Hartman, C. A., Peterson, S. H., Evers, D. C., Jackson, A. K., Elliott, J. E., Vander Pol, S. S. & Bryan, C. E. 2016. Avian mercury exposure and toxicological risk across western North America: A synthesis. *Science of The Total Environment,* 568**,** 749-769. https://doi.org/10.1016/j.scitotenv.2016.03.071

Albert, C. A., D., W. T., Morrissey, C. A., Lai, V. W. M., Cullen, W. R. & Elliot, J. E. 2009. Dose-dependent uptake, elimination, and toxicity of monosodium methanearsonate in adult zebra finches (*Taeniopygia guttata*). *Environmental Toxicology and Chemistry,* 27**,** 605-611. https://doi.org/10.1897/07-147.1

Avery, S. V. 1996. Fate of caesium in the environment: distribution between abiotic and biotic components of aquatic and terrestrial ecosystems. *Journal of Environmental Radioactivity,* 30**,** 139-171. https://doi.org/10.1016/0265-931X(96)89276-9

Baeza, A., del Rio, M., Miro, C., Moreno, A., Navarro, E., Paniagua, J. M. & Peris, M. A. 1991. Radiocesium and radiostrontium levels in song thrushes (*Turdus philomelos*) captured in two regions of Spain. *Journal of Environmental Radioactivity,* 13**,** 13-23. https://doi.org/10.1016/0265-931X(91)90036-F

Bates, D., Maechler, M., Bolker, B. & Walker, S. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software,* 67**,** 1-48. https://doi.org/10.18637/jss.v067.i01

Beaugelin-Seiller, K., Garnier-Laplace, J. & Beresford, N. A. 2020. Estimating radiological exposure of wildlife in the field. *Journal of Environmental Radioactivity,* 211**,** 105830. https://doi.org/10.1016/j.jenvrad.2018.10.006

Beck, M. L., Hopkins, W. A., Hallagan, J. F., Jackson, B. P. & Hawley, D. M. 2014. Exposure to residual concentrations of elements from a remediated coal fly ash spill does not adversely influence stress and immune responses of nestling tree swallows. *Conservation Physiology,* 2**,** cou018. https://doi.org/10.1093/conphys/cou018

Beresford, N. A., Gaschak, S., Maksimenko, A. & Wood, M. D. 2016. The transfer of ^{137}Cs, Pu isotopes, and ^{90}Sr to bird, bat and ground-dwelling small mammal species within the Chernobyl exclusion zone. *Journal of Environmental Radioactivity,* 153**,** 231-236. https://doi.org/10.1016/j.jenvrad.2015.12.027

Bolker, B. 2008. *Ecological models and data in R*, Princeton University Press.

Bondarkov, M. D., Maksimenko, A. M., Gaschak, S. P., Zheltonozhsky, V. A., Jannik, G. T. & Farfán, E. B. 2011. Method for simultaneous ^{90}Sr and ^{137}Cs in-vivo measurements of small animals and other environmental media developed for the conditions of the Chernobyl Exclusion Zone. . *Health Physics,* 101. https://doi.org/10.1097/HP.0b013e318224bb2b

Brandt, J. E., Simonin, M., Di Giulio, R. T. & Bernhardt, E. S. 2019. Beyond selenium: Coal combustion residuals lead to multielement enrichment in receiving lake food webs. *Environmental Science and Technology,* 53**,** 4119-4127. https://doi.org/10.1021/acs.est.9b00324

Brisbin Jr., I. L. 1991. Avian radioecology. *Current Ornithology*, 69-140.
Bryan, A. L., Jr., Hopkins, W. A., Baionno, J. A. & Jackson, B. P. 2003. Maternal transfer of contaminants to eggs in common grackles (*Quiscalus quiscala*) nesting on coal fly ash basins. *Archives of Environmental Contamination and Toxicology,* 45**,** 273-277. https://doi.org/10.1007/s00244-002-0212-9
Carlon, C., Hope, B. & Quercia, F. 2009. Contaminated land: A multi-dimensional problem. *In:* A., M., Suter II, G. W. & Critto, A. (eds.) *Decision Support Systems for Risk-Based Management of Contaminated Sites.* Springer Science & Business Media.
Carlton, W. H., Bauer, L. R., Evans, A. G., Geary, L. A., Murphy Jr., C. E., Pinder, J. E. & Strom, R. N. 1992. Cesium in the Savannah River Site environment. U.S. Department of Energy.
Chapman, M., Dharmarajan, G., Bryan Jr., A. L., DeVault, T. L., Lee, L. & Rhodes Jr., O. E. 2022. Understanding the role of biotic vectors in the accumulation and spread of radioactive contamination on the Savannah River Site. *Waste Management Symposium.* Phoenix, Arizona, USA.
Coates, P. 2013. From hazard to habitat (or hazardous habitat): The lively and lethal afterlife of Rocky Flats, Colorado. *Progress in Physical Geography: Earth and Environment,* 38. https://doi.org/10.1177/030913331351329
Cooper, Z., Bringolf, R., Cooper, R., Loftis, K., Bryan, A. L. & Martin, J. A. 2017. Heavy metal bioaccumulation in two passerines with differing migration strategies. *Science of The Total Environment,* 592**,** 25-32. https://doi.org/10.1016/j.scitotenv.2017.03.055
Currie, L. A. 1968. Limits for qualitative detection and quantitative determination. *Analytical Chemistry,* 40**,** 586-593. https://doi.org/10.1021/ac60259a007
Deonarine, A., Schwartz, G. E. & Ruhl, L. S. 2023. Environmental impacts of coal combustion residuals: Current understanding and future perspectives. *Environmental Science and Technology,* 57**,** 1855-1869. https://doi.org/10.1021/acs.est.2c06094
Dwivedi, D., Steefel, C. I., Arora, B., Banfield, J., Bargar, J., Boyanov, M. I., Brooks, S. C., Chen, X., Hubbard, S. S., Kaplan, D., Kemner, K. M., Nico, P. S., O'Loughlin, E. J., Pierce, E. M., Painter, S. L., Scheibe, T. D., Wainwright, H. M., Williams, K. H. & Zavarin, M. 2022. From legacy contamination to watershed systems science: a review of scientific insights and technologies developed through DOE-supported research in water and energy security. *Environmental Research,* 17. https://doi.org/10.1088/1748-9326/ac59a9
Ehrlich, P., Dobkin, D. S. & Wheye, D. 1988. *Birder's Handbook*, Simon and Schuster.
Eisler, R. 2000. *Handbook of chemical risk assessment: Health hazards to humans, plants, and animals,* Boca Raton, FL, Lewis Publishers.
El Mehdawi, A. F. & Pilon-Smits, E. A. H. 2011. Ecological aspects of plant selenium hyperaccumulation. *Plant Biology,* 14**,** 1-10. https://doi.org/10.1111/j.1438-8677.2011.00535.x
Evers, D. C., Han, Y., Driscoll, C. T., Kamman, N. C., Goodale, M. W., Lambert, K. F., Holsen, T. M., Chen, C. Y., Clair, T. A. & Butler, T. 2007. Biological mercury hotspots in the Northeastern United States and Southeastern Canada. *BioScience,* 57. https://doi.org/10.1641/B570107
Fernández-Martínez, A. & Charlet, L. 2009. Selenium environmental cycling and bioavailability: a structural chemist point of view. *Reviews of Environmental Science and Biotechnology,* 8**,** 81-110. https://doi.org/10.1007/s11157-009-9145-3
Fletcher, D. E., Lindell, A. H., Seaman, J. C., Stankus, P. T., Fletcher, N. D., Barton, C. D., Biemiller, R. A. & McArthur, J. V. 2019. Sediment and biota trace element distribution in streams disturbed by upland industrial activity. *Environmental Toxicology and Chemistry,* 38**,** 115-131. https://doi.org/10.1002/etc.4287
Fletcher, D. E., Lindell, A. H., Stillings, G. K., Blas, S. A. & McArthur, J. V. 2017. Trace element accumulation in lotic dragonfly nymphs: Genus matters. *PLoS ONE,* 12**,** e0172016. https://doi.org/10.1371/journal.pone.0172016

Fletcher, D. E., Lindell, A. H., Stillings, G. K., Mills, G. L., Blas, S. A. & McAurthur, J. V. 2014. Spatial and taxonomic variation in trace element bioaccumulation in two herbivores from a coal combustion waste contaminated stream. *Ecotoxicology and Environmental Safety,* 101.

Gerson, A. R., Fan, R., Qian, G., Schumann, R. C., Olin, P., Howard, D. L. & Smart, R. S. C. 2022. Examination of multiple sources of selenium release from coal wastes and strategies for remediation. *Journal of Hazardous Materials,* 422. https://doi.org/10.1016/j.jhazmat.2021.126924

Gerson, J. R., Walters, D. M., Eagles-Smith, C. A., Bernhardt, E. S. & Brandt, J. E. 2020. Do two wrongs make a right? Persistent uncertainties regarding environmental selenium-mercury interactions. *Environmental Science and Technology,* 54**,** 9228-9234. https://dx.doi.org/10.1021/acs.est.0c01894

Guzy, M. J. & Ritchison, G. 2020. Common Yellowthroat (*Geothylpis trichas*). *In:* Rodewald, P. G. (ed.) *Birds of the World.* version 1.0 ed. Ithaca, NY, USA: Cornell Lab of Ornithology.

Hale, R. & Swearer, S. E. 2016. Ecological traps: current evidence and future directions. *Proceedings of the Royal Society B,* 283**,** 1-8. https://doi.org/10.1098/rspb.2015.2647

Hartig, F. 2022. DHARMa: Residual diagnostics for hierarchical (multi-level/mixed) regression models.

Haskins, D. L., Hamilton, M. T., Jones, A. L., Finger Jr., J. W., Bringolf, R. B. & Tuberville, T. D. 2017. Accumulation of coal combustion residues and their immunological effects in the yellow-bellied slider (*Trachemys scripta scripta*). *Environmental Pollution,* 224**,** 810-819. https://doi.org/10.1016/j.envpol.2017.01.048

Holland, K. E. 2024. *Bioaccumulation and community composition of small terrestrial biota exposed to coal combustion residual contaminants.* Master of Science, University of Georgia.

Hopkins, W. A., Staub, B. P., Baionno, J. A., Jackson, B. P. & Talent, L. G. 2005. Transfer of selenium from prey to predators in a simulated terrestrial food chain. *Environmental Pollution,* 134**,** 447-456. https://doi.org/10.1016/j.envpol.2004.09.010

Hu, Q., Weng, J. & Wang, J. 2010. Sources of anthropogenic radionuclides in the environment: a review. *Journal of Environmental Radioactivity,* 101**,** 426-437. https://doi.org/10.1016/j.jenvrad.2008.08.004

Johnson, R. R., Brown, B. T., Haight, L. T. & Simpson, J. M. 1981. Playback recordings as a special avian censusing technique. *Studies in Avian Biology,* 6**,** 68-75.

Kapustka, L. A. 2004. Issue paper on the ecological effects of metals.: US Environmental Protection Agency.

Kennamer, R. A., Brisbin, J., I. L. , McCreedy, C. K. & Burger, J. 1998. Radiocesium in mourning doves: Effects of a contaminated reservoir drawdown and risk to human consumers. *The Journal of Wildlife Management,* 62**,** 497-508. https://www.jstor.org/stable/3892323

Kennamer, R. A., MCreedy, C. D. & Brisbin, J., I. L. 1993. Patterns of radiocesium contamination in eggs of free-ranging wood ducks. *The Journal of Wildlife Management,* 57**,** 716-724. https://doi.org/10.2307/3809071

Kennamer, R. A., Oldenkamp, R. E., Leaphart, J. C., King, J. D., Bryan Jr., A. L. & Beasley, J. C. 2017. Radiocesium in migratory aquatic game birds using contaminated U.S. Department of Energy reactor-cooling reservoirs: A long-term perspective. *Journal of Environmental Radioactivity,* 171**,** 189-199. http://dx.doi.org/10.1016/j.jenvrad.2017.02.022

Klerks, P. L. & Levinton, J. S. 1989. Effects of heavy metals in a polluted aquatic ecosystem. *In:* Levin, S. A., Kelly, J. R., Harwell, M. A. & Kimball, K. D. (eds.) *Ecotoxicology: Problems and Approaches.* New York, NY: Springer.

Krivolutski, D. A., Lebedeva, K. V. & Shuktomova, I. I. 1999. Birds as objects in bioindication of radioactive pollution. *Acta Biologica Hungarica,* 50**,** 145-160. https://doi.org/10.1007/BF03543038

Latta, S. C., Marshall, L. C., Frantz, M. W. & Toms, J. D. 2015. Evidence from two shale regions that a riparian songbird accumulates metals associated with hydraulic fracturing. *Ecosphere,* 6**,** 144. http://dx.doi.org/10.1890/

ES14-00406.1
Laughlin, A. J., Hudson, T. B. & Brewer-Jensen, T. 2022. Dynamics of an urban chimney swift (*Chaetura pelagica*) roost system during autumn migration. *The Wilson Journal of Ornithology,* 134**,** 269-277. https://doi.org/10.1676/21-00081
Leaphart, J. C., Wilms, K. C., Bryan Jr., A. L. & Beasley, J. C. 2019. Bioaccumulation of 137Cs in anuran larvae utilizing a contaminated effluent canal on the U.S. Department of Energy's Savannah River Site. *Journal of Environmental Radioactivity,* 203**,** 25-29. https://doi.org/10.1016/j.jenvrad.2019.02.012
Leonzio, C., Bianchi, N., Gustin, M., Sorace, A. & Ancora, S. 2009. Mercury, lead and copper in feathers and excreta of small passerine species in relation to foraging guilds and age of feathers. *Bulletin of Environmental Contamination and Toxicology,* 83**,** 693-697. https://doi.org/10.1007/s00128-009-9789-2
Lonvik, K. & Thingstad, P. G. 1999. Radiocaesium accumulation from Chernobyl fallout in nestlings of two pied flycatcher populations (aves) in central Norway; estimating ecological timelag reponses and transfer mechanisms. *Journal of Environmental Radioactivity,* 46**,** 153-169. https://doi.org/10.1016/S0265-931X(98)00122-2
Marques, J. T., Pereira, M. J. R., Marques, T. A., Santos, C. D., Santana, J., Beja, P. & Palmeirim, J. M. 2013. Optimizing sampling design to deal with mist-net avoidance in Amazonian birds and bats. *PLoS ONE,* 8**,** e74505. https://doi.org/10.1371/journal.pone.0074505
Mattsson, B. J. & Cooper, R. J. 2006. Louisiana waterthrushes (*Seiurus motacilla*) and habitat assessments as cost-effective indicators of instream biotic integrity. *Freshwater Biology,* 51**,** 1941-1958. https://www.doi.org/10.1111/j.1365-2427.2006.01617.x
Mattsson, B. J. & Cooper, R. J. 2009. Multiscale analysis of the effects of rainfall extremes on reproduction by an obligate riparian bird in urban and rural landscapes. *Auk,* 126**,** 64-76. https://doi.org/10.1525/auk.2009.08001
Mattsson, B. J., Master, T. L., Mulvihill, R. S. & Robinson, W. D. 2020. Louisiana Waterthrush (*Parkesia motacilla*). *In:* Poole, A. F. (ed.) *Birds of the World.* version 1.0 ed. Ithaca, NY, USA: Cornell Lab of Ornithology.
Meyer, C. B., Schlekat, T. H., Walls, S. J., Iannuzzi, J. & Souza, M. J. 2014. Evaluating risks to wildlife from coal fly ash incorporating recent advances in metals and metalloids risk assessment. *Integrated Environment Assessment and Management,* 11**,** 67-79. https://doi.org/10.1002/ieam.1576
Nesbitt, S. A., Harris, B. A., Repenning, R. W. & Brownsmith, C. B. 1982. Notes on Red-Cockaded Woodpecker Study Tecnhiques. *Wildlife Society Bulletin,* 10**,** 160-163. https://www.jstor.org/stable/3781737
Ohlendorf, H. M. & Heinz, G. H. 2011. Selenium in birds. *In:* Beyer, W. N. & Meador, J. P. (eds.) *Environmental Contaminants in Biota: Interpreting Tissue Concentrations.* 2 ed. Boca Raton, FL, USA: CRC.
Ohlendorf, H. M., Kilness, A. W., Simmons, J. L., Stroud, R. K., Hoffman, D. J. & Moore, J. F. 1988. Selenium toxicosis in wild aquatic birds. *Journal of Toxicology and Environmental Health,* 24**,** 67-92. https://doi.org/10.1080/15287398809531141
Oldenkamp, R. E., Bryan Jr., A. L., Kennamer, R. A., Leaphart, J. C., Webster, S. C. & Beasley, J. C. 2017. Trace elements and radiocesium in game species near contaminated sites. *The Journal of Wildlife Management,* 81**,** 1338-1350. https://doi.org/10.1002/jwmg.21314
Palmer, E. 1997. Preliminary report on coal pile, coal pile runoff basins, and ash basins at the Savannah River Site: effects on groundwater.
Pigot, A. L., Sheard, C., Miller, E. T., Bregman, T. P., Freeman, B. G., Roll, U., Seddon, N., Trisos, C. H., Weeks, B. C. & Tobias, J. A. 2020. Macroevolutionary convergence connects morphological form to ecological function in birds. *Nature Ecology & Evolution,* 4**,** 230-239. https://doi.org/10.1038/s41559-019-1070-4

Presser, T. S. & Luoma, S. N. 2010. A methodology for ecosystem-scale modeling of selenium. *Integrated Environment Assessment and Management,* 6**,** 685-710. https://doi.org/10.1002/ieam.101

Puls, R. 1988. *Mineral levels in animal health: Diagnostic data,* Clearbrook, British Columbia, Sherpa International.

Rafiei, M. M., Tavakoli-Anbaran, H. & Kurudirek, M. 2020. A detailed investigation of gamma-ray energy absorption and dose buildup factor for soft tissue and tissue equivalents using Monte Carlo simulation. *Radiation Physics and Chemistry,* 177. https://doi.org/10.1016/j.radphyschem.2020.109118

Rosenberg, K. V., Dokter, A. M., Blancher, P. J., Sauer, J. R., Smith, A. C., Smith, P. A., Stanton, J. C., Panjabi, A., Helft, L., Parr, M. & Marra, P. P. 2019. Decline of the North American avifauna. *Science,* 366**,** 120-124. https//www.doi.org/10.1126/science.aaw1313

Sample, B. E. & Irvine, C. 2011. Radionuclides in biota. *Environmental Contaminants in Biota.* CRC Press.

Sánchez-Virosta, P., Espín, S., García-Fernández, A. J. & Eeva, T. 2015. A review on exposure and effects of arsenic in passerine birds. *Science of The Total Environment,* 512**,** 506-525. https://doi.org/10.1016/j.scitotenv.2015.01.069

Scheuhammer, A. M. 1987. The chronic toxicity of aluminium, cadmium, mercury, and lead in birds: A review. *Environmental Pollution,* 46**,** 263-295. https://doi.org/10.1016/0269-7491(87)90173-4

Schlichting, P. E., Dombrovski, V. C. & Beasley, J. C. 2020. Use of abandoned structures by Przewalski's wild horses and other wildlife in the Chernobyl Exclusion Zone. *Mammal Research,* 65**,** 161-165. https://doi.org/10.1007/s13364-019-00451-4

Seewagen, C. L. & Newhouse, M. 2018. Mass changes and energetic condition of grassland and shrubland songbirds during autumn stopovers at a reclaimed landfill in the New Jersey meadowlands. *The Wilson Journal of Ornithology,* 130**,** 377-384. https://doi.org/10.1080/00063657.2021.1960269

Sievers, M., Hale, R., Parris, K. M. & Swearer, S. E. 2018. Impacts of human-induced environmental change in wetlands on aquatic animals. *Biological Reviews,* 93**,** 529-554. https://doi.org/10.1111/brv.12358

Silva, A. E., Speakman, R. J., Barns, B. F., Coyle, D. R., Leaphart, J. C., Abernethy, E. F., Turner, K. L., Rhodes Jr., O. E., Beasley, J. C. & Gandhi, K. J. K. 2023. Bioaccumulation of contaminants in Scarabidae and Silphidae beetles at sites polluted by coal combustion residuals and radiocesium. *Science of The Total Environment,* 904**,** 166821. https://doi.org/10.1016/j.scitotenv.2023.166821

SRNS. 2020. Comprehensive alternative evaluation of ash and coal fine waste units at the Savannah River Site.

Stanley, T. R., Jr., Spann, J. W., Smith, G. J. & Rosscoe, R. 1994. Main and interactive effects of arsenic and selenium on mallard reproduction and duckling growth and survival. *Archives of Environmental Contamination and Toxicology,* 26**,** 444-451. https://doi.org/10.1007/BF00214145

Sternalski, A., Matsui, S., Bonzom, J. M., Kasahara, S., Beaugelin-Seiller, K., Ueda, K., Watanabe, M. & Adam-Guillermin, C. 2015. Assessment of radiological dose rates in passerine birds after the Fukushima nuclear accident according to their life stage. *Japanese Journal of Ornithology,* 64**,** 161-168. https://doi.org/10.3838/jjo.64.161

Stewart, R. E. 1953. A Life History Study of the Yellow-Throat. *The Wilson Bulletin,* 65**,** 99-115. https://www.jstor.org/stable/4158186

Thomas, I., J. C., Kieran, T. J., Finger Jr., J. W., Bayona-Vásquez, N. J., Oladeinde, A., Beasley, J. C., Seaman, J. C., McArthur, J. V., Rhodes Jr., O. E. & Glenn, T. C. 2021. Unveiling the gut microbiota and resistome of wild cotton mice, *Peromyscus gossypinus*, from heavy metal- and radionuclide-contaminated sites in the southeastern United States. *Environmental Microbiology,* 9**,** e00097-21. https://doi.org/10.1128/spectrum.00097-21

Tobias et al., J. A. 2022. AVONET: Morphological, ecological and geographical data for all birds. *Ecology Letters,* 25**,** 581-597. https://doi.org/10.1111/ele.13898

Tsipoura, N., Burger, J., Feltes, R., Yacabucci, J., Mizrahi, D., Jeitner, C. & Gochfeld, M. 2008. Metal concentrations in three species of passerine birds breeding in the Hackensack Meadowlands of New Jersey. *Environmental Research,* 107**,** 218-228. https://doi.org/10.1016/j.envres.2007.11.003

USEPA. 2011. Final Determination of the U.S. Environmental Protection Agency Pursuant to § 404(c) of the Clean Water Act Concerning the Spruce No. 1 Mine, Logan County, West Virginia.

USEPA. 2024. Institutional and engineering controls data. https://rcrapublic.epa.gov/rcrainfoweb/action/modules/cor/caindex

Walls, S. J., Meyer, C. B., Iannuzzi, J. & Schlekat, T. H. 2014. Effects of coal fly ash on tree swallow reproduction in Watts Bar Reservoir, Tennessee. *Integrated Environment Assessment and Management,* 11. 10.1002/ieam.1585

Werner, C. S., Chapman, M., Skaggs, J., Rhodes Jr., O. E. & DeVault, T. L. 2024. Trace metal transfer to passerines inhabiting wastewater treatment wetlands. *Science of The Total Environment,* 927**,** 172373. https://doi.org/10.1016/j.scitotenv.2024.172373

White, D. L. & Gaines, K. F. 2000. The Savannah River Site: Site description, land use and management history. *Avian Research at the Savannah River Site: A Model for Integrating Basic Research and Long-Term Management.* Scholarly Commons.

Table 2.1. Bird species captured on the Savannah River Site across CCR, NFP, and reference sites, with sample sizes. Each species was assigned a trophic category according to the proportion of their annual diet obtained from various trophic levels (Tobias et al. 2022; Pigot et al. 2020) and a ground score according to their tendency to nest and/or forage on the ground (Ehlrich 1988).

Trophic category	Ground score	Species	N
Herbivore	0	Indigo Bunting (*Passerina cyanea*)	1
	1	Northern cardinal (*Cardinalis cardinalis*)	37
		Red-winged blackbird (*Agelaius phoeniceus*)	8
	2	Eastern towhee (*Pipilo erythrophthalmus*)	2
Omnivore	0	Red-eyed vireo (*Vireo olivaceus*)	20
		Great-crested flycatcher (*Myiarchus crinitus*)	18
		Carolina chickadee (*Poecile carolinensis*)	14
		Red-bellied woodpecker (*Melanerpes carolinus*)	4
	1	Gray catbird (*Dumetella carolinensis*)	2
		Northern mockingbird (*Mimus polyglottus*)	2
		Wood thrush (*Hylocichla mustelina*)	1
		Blue jay (*Cyanocitta cristata*)	1
	2	Swamp sparrow (*Melospiza georgiana*)	9
		Hermit thrush (*Catharus guttatus*)	1
Carnivore	0	White-eyed vireo (*Vireo griseus*)	58
		Tufted titmouse (*Baeolophus bicolor*)	32
		Hooded warbler (*Setophaga citrina*)	16
		Summer tanager (*Piranga rubra*)	14
		Pine warbler (*Setophaga pinus*)	13
		Downy woodpecker (*Picoides pubescens*)	11
		Northern parula (*Setophaga americana*)	6
		Acadian flycatcher (*Empidonax virescens*)	8
		Prothonotary warbler (*Protonotaria citrea*)	5
		Eastern bluebird (*Sialia sialis*)	4
		Eastern wood pewee (*Contopus virens*)	4
		Yellow-throated vireo (*Vireo flavifrons*)	3
		Hairy woodpecker (*Leuconotopicus villosus*)	2
		Red-headed woodpecker (*Melanerpes erythrocephalus*)	2
		Eastern kingbird (*Tyrannus tyrannus*)	1
		Orchard oriole (*Icterus spurius*)	1
		Pileated woodpecker (*Dryocopus pileatus*)	1
	1	Common yellowthroat (*Geothylpis trichas*)	41
		Common grackle (*Quiscalus quiscula*)	11
		Black-and-white warbler (*Mniotilta varia*)	10
		Blue grosbeak (*Passerina caerulea*)	5
		Eastern phoebe (*Sayornis phoebe*)	4
	2	Carolina wren (*Thryothorus ludovicianus*)	39
		Louisiana waterthrush (*Parkesia motacilla*)	15
		Kentucky warbler (*Geothylpis formosa*)	4

Table 2.2. Generalized linear mixed effects models showing predictors of blood Se, Hg, and As concentrations and whole-body ^{137}Cs activity concentrations in passerines on the Savannah River Site. The best-fitting model, determined by the lowest AIC, is reported for each element. The inverse link function was applied to all coefficients and standard errors. Therefore, exponentiated coefficients for Se and Hg are interpreted multiplicatively to derive expected blood concentration (ppm *ww*) for a set of predictors, whereas inverse logit coefficients for As and ^{137}Cs are interpreted additively to derive the probability of detection.

Selenium

Se ~ contaminant history + trophic category + (1|species), family = gaussian(link = "log")

Fixed Effect	Coefficient	Std. Err.	t value	p value
Intercept	2.82	1.18	6.36	<0.001
CCR	1.31	1.04	6.70	<0.001
Carnivore	1.51	1.18	2.42	0.02
Omnivore	1.49	1.20	2.15	0.03

Random Effect	Variance	Std. Dev.
Species	0.16	0.40
Residual	3.32	1.82

Mercury

Hg ~ contaminant history + trophic category + contaminant history*trophic category + (1|species), family = gamma(link = "log")

Fixed Effect	Coefficient	Std. Err.	t value	p value
Intercept	0.23	1.89	-2.30	0.02
CCR	1.47	1.19	2.24	0.02
Carnivore	2.12	1.97	1.11	0.27
Omnivore	1.58	2.09	0.62	0.53
CCR: Carnivore	0.72	1.22	-1.66	0.10
CCR: Omnivore	0.44	1.30	-3.18	0.001

Random Effect	Variance	Std. Dev.
Species	0.38	0.62
Residual	0.25	0.50

Arsenic

As ~ contaminant history + ground + contaminant history*ground + (1|species), family = binomial(link = "logit")

Fixed Effect	Coefficient	Std. Err.	t value	p value
Intercept	-0.04	0.63	-5.89	<0.001
CCR	0.75	0.63	1.98	0.048
Ground	0.75	0.60	2.61	0.009
CCR:Ground	-0.31	0.61	-1.71	0.09

Random Effect	Variance	Std. Dev.
Species	0.60	0.77

Cesium-137

^{137}Cs ~ contaminant history + ground + detector + (1|species), family = binomial(link = "logit")

Fixed Effect	Coefficient	Std. Err.	t value	p value
Intercept	-0.19	0.72	-1.54	0.12
NFP	0.83	0.64	2.68	0.007
Ground	0.71	0.62	1.85	0.06
Detector	-0.20	0.65	-2.18	0.03

Random Effect	Variance	Std. Dev.
Species	0.83	0.91

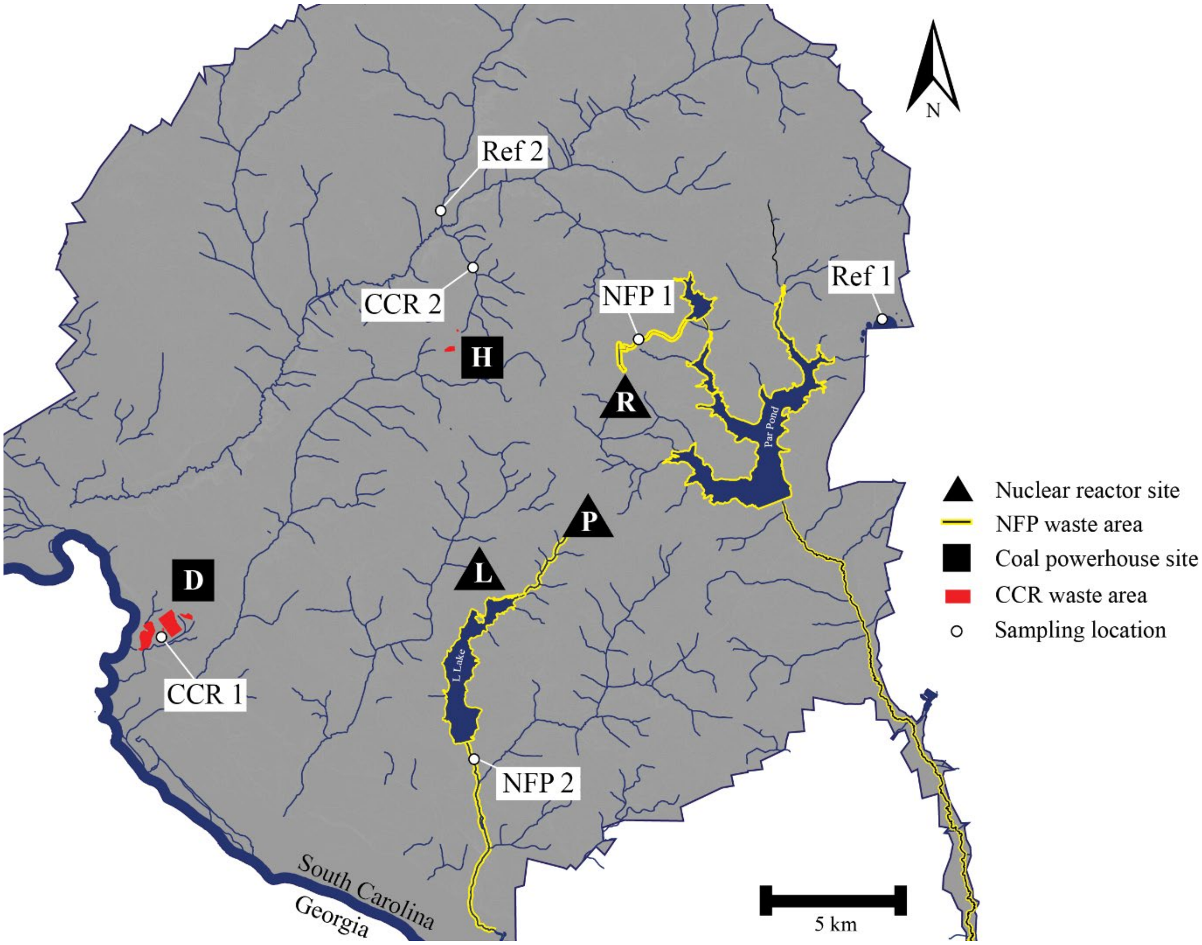

Figure 2.1. Map of the U.S. Department of Energy's Savannah River Site in South Carolina. Passerine sampling occurred along Beaver Dam Creek and a nearby retention pond (CCR 1) and along McQueen's Branch (CCR 2), which are affected by CCR from the D-Area and H-Area ash basins, respectively. Sampling also occurred along R Canal (NFP 1) and Steel Creek (NFP 2) which are contaminated with NFP from the R-, L-, and P-area reactors. Craig's Pond (Ref 1) and Upper Three Runs/Tinker Creek (Ref 2) served as uncontaminated reference areas.

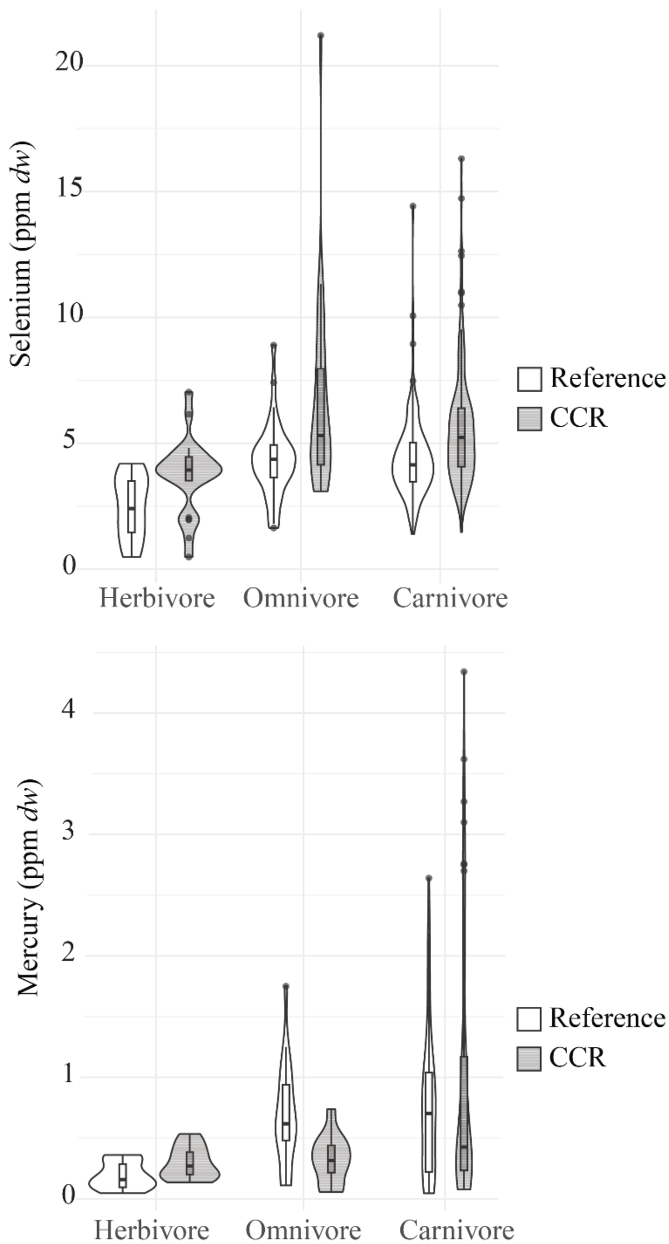

Figure 2.2. The distribution of blood Se and Hg concentrations (ppm *dw*) in passerines across trophic categories at reference and CCR sites. The width of each violin plot indicates the density of observations for a given element concentration.

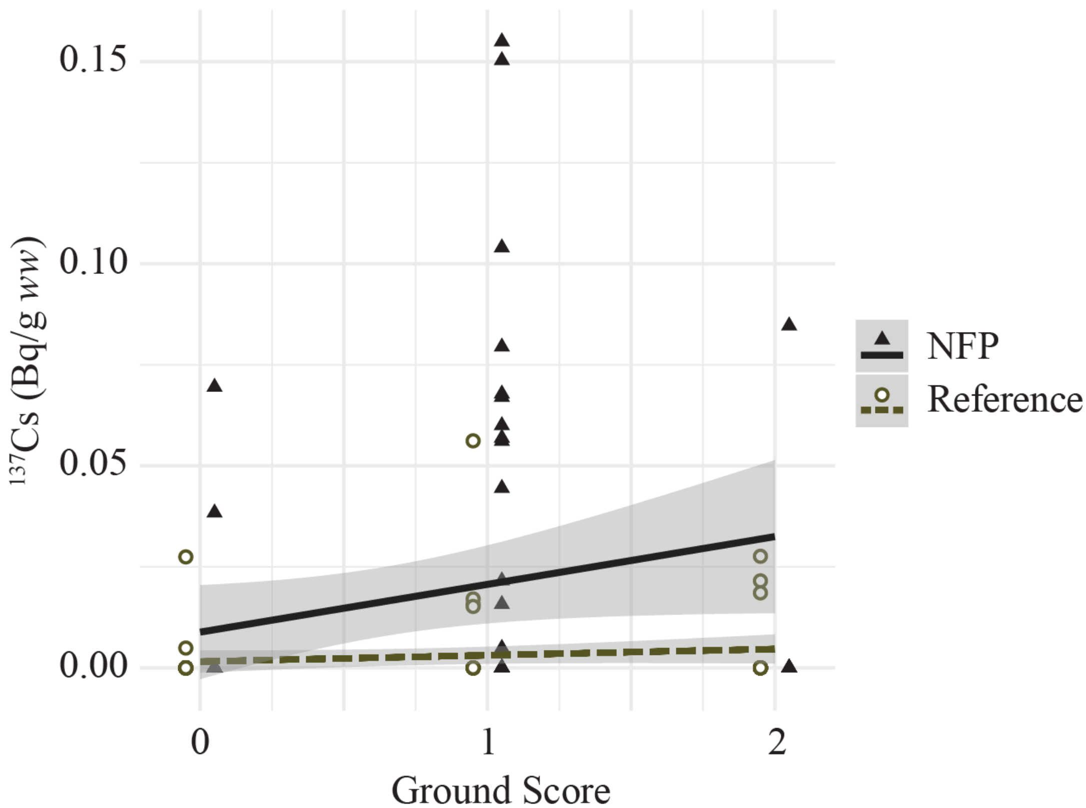

Figure 2.3. The relationship between ^{137}Cs activity concentrations and ground score in passerines at reference and NFP sites, with linear regression lines and 95% confidence intervals. A higher ground score indicates a tendency to spend more time on the ground via foraging and/or nesting.

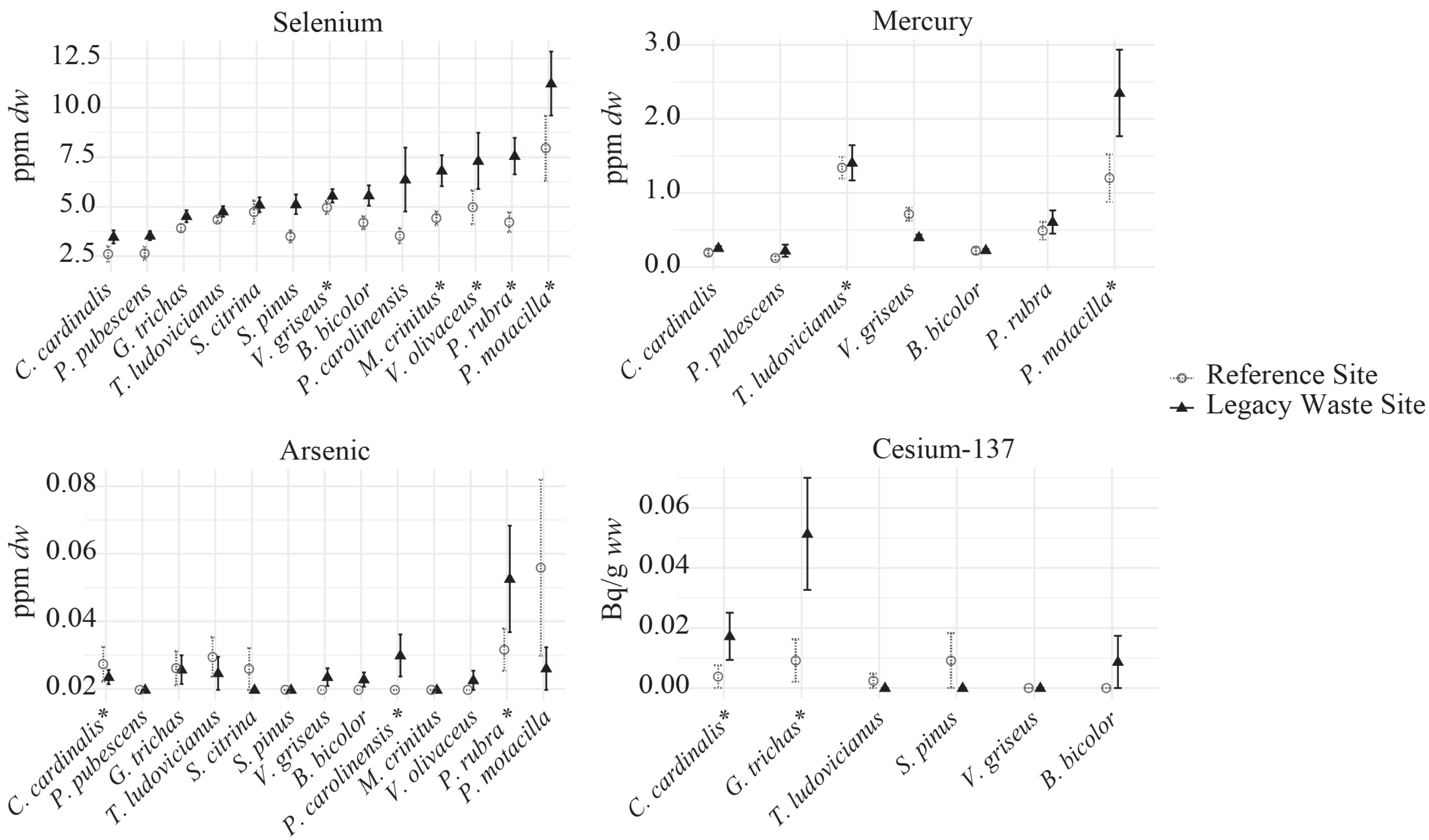

Figure 2.4. Mean blood Se, Hg, and As concentrations (ppm *dw*) or whole-body ^{137}Cs activity concentrations (Bq/g *ww*) for the most abundant passerine species (n ≥ 3 per site type) at reference and legacy waste sites. Error bars represent standard errors. Species marked with asterisks had significantly higher element concentrations than was predicted by site contaminant history, trophic category, and ground score, as indicated by the conditional means of the random effect.

CHAPTER 3

USE OF CONTAMINATED HABITAT AND ASSOCIATED SELENIUM UPTAKE MEDIATE HAEMOSPORIDIAN PARASITE INFECTION IN WILD PASSERINE BIRDS

Werner,C.S., Chapman, M., Peach, D.A.H., DeVault, T.L., and Rhodes Jr., O.E. Submitted to *Ecology and Evolution,* 2025.

ABSTRACT

Environmental contamination alters ecological interactions among organisms, including those associated with parasitism. Contaminants can mediate parasitic relationships at multiple scales by changing host vulnerability to infection and disrupting transmission-relevant contacts. The overall effect of contamination on parasitism remains poorly understood, yet the interplay between these stressors has significant implications for animal and human health. We conducted a community-scale field study to evaluate whether trace element contaminants derived from coal combustion residuals and nuclear fission products alter the dynamics of haemosporidian blood parasites, dipteran vectors, and avian hosts in riparian and wetland habitats in South Carolina, USA. We captured 329 individuals of 31 passerine bird species and 195 *Culex* mosquito vectors at two sites affected by coal combustion waste, two sites affected by nuclear fission waste, and two reference sites. We evaluated whether blood concentrations of zinc, copper, lead, mercury, selenium, and arsenic and whole-body radioactivity concentrations due to cesium-137 predicted the likelihood of single and coinfections by *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* within passerine hosts. We also evaluated whether the likelihood of haemosporidian infection in birds and *Plasmodium* infection in *Culex* vectors differed with the presence of site-level contamination. Individual passerine hosts inhabiting coal combustion waste sites had significantly higher blood selenium concentrations than those at reference sites, and blood selenium was negatively associated with the likelihood of *Leucocytozoon* infection. At the site level, coal combustion waste was associated with higher incidences of *Haemoproteus* and *Leucocytozoon* among host populations. *Plasmodium* did not differ in hosts or vectors across sites. The transfer of low-dose, waste-derived selenium to wildlife may bolster individual response to some parasites and increase the reservoir capacity of host populations. Our findings highlight complex effects of trace elements on wildlife disease dynamics and reveal priorities for future research in contaminated habitat.

INTRODUCTION

Anthropogenic waste fundamentally alters the chemistry of Earth's ecosystems (UNEP, 2020). Processes associated with mining, coal combustion, hydraulic fracturing, and nuclear fission generate byproducts that contain metals, metalloids, and radionuclides, many of which occur naturally in trace amounts but are released to the environment in excess concentrations or new forms (Deonarine et al., 2023, Dwivedi et al., 2022, Estrada and Bhamidimarri, 2016). Influxes of waste-derived elements transform ecosystem properties by creating novel chemical environments in which living organisms interact (Kapustka, 2004). Chemical stressors alter the movement, nutrition, reproduction, and immune function of organisms and exert selective pressures on populations, with consequences for ecosystem function and human and animal health (Kramer et al., 2010, Saaristo et al., 2018).

Parasites, vectors, and hosts maintain dynamic ecological relationships that are affected by disturbances to their shared environments (Morales-Castilla et al., 2021, Patz et al., 2000, Werner and Nunn, 2020). Trace metal or radionuclide contamination can alter these relationships at multiple scales, with multifaceted effects on parasite transmission (Lafferty and Kuris, 1999, Marcogliese, 2005). One possible outcome of such alterations is greater parasitism among hosts inhabiting contaminated environments. For instance, exposure to excess trace elements or ionizing radiation can induce oxidative stress and immunosuppression (Becker et al., 2017, Kesäniemi et al., 2019, Koller, 1980), thereby increasing host vulnerability to parasite infection (Morley et al., 2006). Suppression of immune defenses may allow a greater number of parasite species to establish infections in a host, with consequences for disease severity (Pigeault et al., 2018). Contaminants can also impair host movement (Kojima et al., 2024), and limit grooming behaviors (Burbacher et al., 1990), resulting in higher rates of exposure to vector-transmitted parasites. At a landscape scale, environmental contamination can degrade habitat quality and reduce food availability for hosts, leading to indirect effects of contaminants on host nutrition, body mass, and immune function (Sánchez et al., 2020). The combined stressors of parasite infection and contaminant exposure may have antagonistic or synergistic effects on host health (Kiesecker, 2002, Marcogliese and

Pietrock, 2011, Morrill et al., 2019). In the latter case, the improper disposal of trace element waste and subsequent contamination of natural resources can exacerbate the severity of disease impacts on vulnerable communities of wildlife, domestic animals, and humans.

Contaminants can also hinder parasite transmission among hosts in polluted habitats. Parasites with complex life cycles, or heteroxenous parasites, rely on the integrity of multiple ecological interactions between their definitive and intermediate hosts or vectors to complete their development. Parasites in this category are estimated to be in decline globally due to biodiversity loss (Dunn et al., 2009; but see Keesing & Ostfeld 2021), and their presence or richness has been proposed as a metric of ecosystem integrity, with diminished parasite communities indicating impaired ecosystem function in disturbed environments (Marcogliese, 2005, Sures et al., 2017). Contaminant-induced changes to host or vector fitness, population size, and movement patterns can suppress parasites with complex life cycles if the pathways integral to their transmission are disrupted (Neff and Dharmarajan, 2021, Sánchez et al., 2020). Many heteroxenous parasites of terrestrial vertebrates undergo partial development within hosts or vectors that have aquatic life stages and are particularly vulnerable to the accumulation of trace element contaminants in freshwater systems (Marcogliese, 2005, Poulin, 1992). Additionally, if definitive hosts experience rapid mortality after contaminant exposure, then host density and transmission-relevant contact rates are likely to decline (Sánchez et al., 2020). The study of heteroxenous parasites in contaminated ecosystems can alert investigators to the loss of their hosts or vectors and serve as an indication of community-scale toxicity impacts (Sures et al., 2017).

Studies investigating the relationships between contaminants, parasites, and hosts have revealed a range of effects, likely due to the contradictory factors that govern these complex relationships in different systems. Prior work has occurred primarily in freshwater environments and has demonstrated that parasitism can increase or decrease in aquatic wildlife exposed to trace element pollution, with the direction and magnitude of effects dependent on system-specific factors like parasite life cycle and contaminant bioavailability (Poulin, 1992, Sures et al., 2017). By comparison, few studies have investigated the effects of trace element pollution on parasitism in terrestrial wildlife (Cable et al., 2022, Riley et al., 2014), and even fewer have incorporated vectors or intermediate hosts when examining how contaminants mediate the host-parasite relationship. Despite this, terrestrial wildlife are known to accumulate contaminants in habitats affected by anthropogenic waste (Ackerman et al., 2016, Becker et al., 2018, Oldenkamp et al., 2017) and serve as primary reservoirs of zoonotic parasites with paramount medical and veterinary importance (Daszak et al., 2000, Miller et al., 2013). Furthermore, the precipitous declines of several terrestrial wildlife species are attributed to either contaminant toxicity (Bowerman et al., 1995, Finkelstein et al., 2012) or disease (Atkinson and Samuel, 2010, Hoyt et al., 2021), and a greater understanding of the interactions between these stressors is necessary for proper management of threatened populations.

Passerine birds are diverse, highly mobile, and often abundant in disturbed environments (Maklakov et al., 2011, Seewagen and Newhouse, 2018). Passerines accumulate heavy metals, metalloids, and radionuclides through trophic transfer and contact with contaminated substrates (Ackerman et al., 2016, Sternalski et al., 2015, Werner et al., 2024) and have long served as bioindicators of environmental pollution (Burger, 1993). Passerines also harbor parasites that have significantly impacted naïve bird species, poultry, and humans (Atkinson and Samuel, 2010, Ayala et al., 2020, Ezenwa et al., 2006). Perhaps the best-studied avian parasites are haemosporidia of the genus *Plasmodium,* which are closely related to the agents of human malaria and are primarily vectored by *Culex* mosquitoes (Valkiunas, 2004). *Plasmodium* in wild birds often co-occur with the haemosporidia *Haemoproteus* and *Leucocytozoon*, which are vectored by biting midges (*Culicoides*), and simuliid flies (Simuliidae), respectively (Valkiunas, 2004). Avian haemosporidia are widely used as models for understanding the interactions between hosts, vectors, and parasites (Ellis et al., 2020, Lotta et al., 2019, Pigeault et al., 2018) and are therefore excellent candidates for evaluating how environmental contamination mediates these relationships.

This study investigated whether trace element contamination alters the interactions between haemosporidian parasites, mosquito vectors, and avian hosts in riparian and wetland habitats. We evaluated whether blood concentrations of four heavy metals and two metalloids – zinc (Zn), copper (Cu), lead (Pb), mercury (Hg), selenium (Se), and arsenic (As) – and whole-body radioactivity concentrations due to the radionuclide cesium-137 (^{137}Cs) predicted the likelihood of single and coinfections by *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* within passerine hosts inhabiting two sites affected by coal combustion waste, two sites affected by nuclear fission waste, and two reference sites. We also evaluated whether the likelihood of *Plasmodium* infection in *Culex* vectors differed with the presence of site-level contamination. We tested two hypotheses that represent the potential, divergent effects of contaminants on vector-transmitted parasites at different ecological scales: (1) sublethal contaminant exposure increases parasitism by fostering favorable conditions for parasite communities within hosts, and (2) environmental contamination decreases parasitism by disrupting the transmission of parasites among host and vector communities. Evidence in support of the first hypothesis would include a higher likelihood of haemosporidian single and/or coinfection in passerine hosts with higher metal, metalloid, or radionuclide burdens. Evidence in support of the second hypothesis would include a lower likelihood of haemosporidian infection in avian hosts and *Culex* vectors at contaminated sites compared to reference sites and in hosts that are year-round residents of contaminated areas compared to those that are recent migrants. We aim to provide a nuanced perspective on how altered environmental chemistry due to industrial waste affects interactions between parasites, vectors, and terrestrial wildlife.

METHODS

Site Description

The Savannah River Site (SRS) is a U.S. Department of Energy facility in the upper coastal plain of South Carolina that historically produced materials for the national defense. Ten percent of the 780 km^2 property is characterized by industrial use, and the remaining area contains managed forests and wetlands (SRNS, 2022). Habitat located upstream of industrial facilities is minimally impacted by site operations, but areas adjacent to and downstream of former nuclear reactors and coal-fired power plants are impacted by legacy wastes from releases of nuclear fission products (NFP) and coal combustion residuals (CCR; Carlton et al., 1992). Ecosystems on the SRS are therefore exposed to varying concentrations of waste-derived trace elements and radionuclides, providing unique opportunities to assess the ecological effects of these contaminants in field settings.

Avian sampling

We captured passerines and *Culex* spp. at six sites on the SRS within riparian and wetland habitats. Craig's Pond (reference 1) and Upper Three Runs (reference 2) are located upstream of point-source contamination and have been preserved as ecological reference areas since 1952 (White and Gaines, 2000). D-Area (CCR 1) and McQueen's Branch (CCR 2) are located within the same drainage basins as coal combustion waste areas that serve as ongoing sources of metals and metalloids to the surrounding ecosystems (SRNS, 2020). R-Canal (NFP 1) and Steel Creek (NFP 2) are freshwater systems that received reactor cooling effluent contaminated with NFP and are primarily affected by the radionuclide ^{137}Cs (Carlton et al., 1992).

We used mist nets to capture passerines during the breeding season from March to July of 2023. We spent two weeks at each site before rotating, ensuring that CCR, NFP, and reference site types were sampled throughout the breeding season. Each of the four reference and CCR sites were visited twice, for a total of four weeks per site. Due to equipment constraints, NFP sites were each visited once, for a total of two weeks per site. At each site, we placed four to ten 30 mm mist nets at the edges of wetlands and streams and in adjacent forested habitat. We opened mist nets from sunrise to approximately 1100 and checked them every 20 minutes. We aimed for a sample that represented the full community of resident and migrant passerines and used song callbacks to obtain even numbers of species across site types.

We measured the body mass of each bird and attached an aluminum USGS band at the time of capture. We then collected 0.07-0.30 mL of blood from the jugular vein using an insulin syringe fitted with a 28-gauge needle. For parasite diagnostics, three to four drops of blood were stored on Whatman FTA cards

(Qiagen), chilled in the field, and transferred to a refrigerator in the lab on the day of capture. The remaining blood was transferred to metal-free vials for trace element analysis.

We quantified whole-body radioactivity concentrations due to ^{137}Cs and blood concentrations of Zn, Cu, Pb, As, Se, and Hg according to methods detailed in Werner et al. (2025). Briefly, we measured the gamma radiation emitted from each live bird for 30 minutes using a high-purity germanium (HPGe) spectrometer (Aegis; Mirion Technologies) attached to a robust Pb shield and mounted within a trailer at our field site. We used the Genie™ 2000 spectroscopy software to locate peaks in the ^{137}Cs region of interest (662 keV) that were significantly above the Critical Level (Lc; $\alpha = 0.05$) calculated according to Currie (1968). If ^{137}Cs was present, its radioactivity concentration in the sample was determined after subtracting background radiation derived from field blanks and applying an efficiency correction tailored to the geometry of our counting system. Radioactivity concentrations are reported in Bq/g wet weight. In the laboratory, we analyzed blood samples for Zn, Cu, Pb, As, and Se using an Inductively-Coupled Plasma Mass Spectrometer NexION 300X (PerkinElmer) and for Hg using a Direct Mercury Analyzer (DMA-80, Milestone Shelton, CT, USA). Reference materials (TORT-3 and DOLT-5) and a blank were included in each set of samples, and method detection limits (MDL) were calculated for each element. Trace element concentrations are reported in ppm dry weight.

Vector sampling

Once every four weeks from April to July, we captured *Culex* spp. at all six sites simultaneously using infusion-based gravid traps designed to attract ovipositing, adult female *Culex* mosquitoes (Williams and Gingrich, 2007). To mirror locally available oviposition substrates, we prepared an oak leaf infusion by steeping one kilogram of dried oak leaves obtained from the SRS in 30 liters of well water for eight days (Allan et al., 2005, O'Meara et al., 1989). Frommer Updraft Gravid Traps (Model 1719, John Hock Company) were filled with five liters of infusion, placed in the field between 1300 and 1800 hours, and picked up 10-16 hours later. Traps containing live mosquitoes were transferred to the laboratory immediately and placed in a −20°C freezer to euthanize mosquitoes.

After euthanasia, we identified gravid *Culex* females using a dissection microscope and a morphological identification key (Burkett-Cadena, 2013). Each *Culex* female was bisected at the junction of the thorax and abdomen using sterilized forceps and scalpel (Foley et al., 2012), and cephalothoraxes were stored at −80°C in 95% ethanol until DNA extraction .

Molecular analysis

Parasite DNA was extracted from 3-mm hole punches of cards containing dried avian blood and from the cephalothoraxes of *Culex* using the DNeasy Blood and Tissue Extraction Kit (Qiagen). We evaluated the presence of *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* in each avian blood sample using a multiplex PCR assay with the primer sets PMF/PMR, HMF/HMR, and LMF/LMR (Ciloglu et al. 2019). Each reaction contained 5 µL of commercial master mix (2x Qiagen Multiplex PCR Master Mix, Qiagen, Hilden, Germany), 0.2 µL of each of the six primers (10 µM), 1.8 µL of nanopure H_2O, and 2 µL of template DNA. An initial denaturation step of 95 ºC for 15 min was followed by 35 cycles of 94 ºC for 30s, 59 ºC for 90s, and 72 ºC for 30s, with a final annealing step at 72 ºC for 10 minutes. Positive and negative controls (H_2O) were included in every PCR run. Amplification products were visualized on 2% agarose gels containing GelRedTM gel stain (Biotium, Inc., Hayward, CA, USA) in a Gel Doc XR+ with Image Lab Software (Bio-Rad, CA, USA). We noted the presence of bands corresponding to *Plasmodium* (378 bp), *Haemoproteus* (533 bp), and/or *Leucocytozoon* (218 bp) infection in each sample.

We evaluated the presence of *Plasmodium* in each *Culex* cephalothorax sample using a nested PCR with the primer pairs HaemNF1/HaemF and HaemNR3/HaemR2 to amplify a 480 bp fragment of the *cytb* gene (Hellgren et al., 2004). Each 10 µL reaction mix contained 5 µL of commercial master mix (2x Qiagen Multiplex PCR Master Mix, Qiagen, Hilden, Germany), 3.1 µL of nanopure H_2O, 0.5 $MgCl_2$, 0.2 µL of each primer (10 µM), and 1 µL of template DNA (25 ng/uL). The first reaction used the primer pairs HaemNFI and HaemF and was carried out with an initial denaturation step of 95 ºC for 15 min followed by 20 cycles of 94 ºC for 30s, 50 ºC for 30s, and 72 ºC for 45s, with a final annealing step at 72 ºC for 10 minutes. The second reaction used 1 µL of PCR product as template DNA and the primers

HaemF and HaemR2. The cycling parameters were the same as the initial reaction but with 35 cycles. Positive and negative controls (H_2O) were included in every PCR run. We visualized amplification products on 2% agarose gels containing GelRedTM gel stain (Biotium, Inc., Hayward, CA, USA) in a Gel Doc XR+ with Image Lab Software (Bio-Rad, CA, USA) to detect the presence of a band corresponding to *Plasmodium.*

Data analysis

All statistical analyses were conducted in R. We used Kruskal-Wallis tests and Dunn post-hoc tests to evaluate whether average concentrations of Zn, Cu, Pb, Se, As, Hg, and ^{137}Cs in birds differed between sites with varying contaminant histories. When a trace element was undetectable in a blood sample, a value equivalent to one half of the instrument method detection limit (MDL) was used for that sample in statistical analysis. When radioactivity due to ^{137}Cs was below the critical level, ^{137}Cs was assumed to be absent and a value of zero was used for that sample.

We used generalized linear mixed effects models (GLMMs) to evaluate whether haemosporidian infection in birds was predicted by (1) site contaminant history, (2) blood concentrations (ppm *dw*) of Zn, Cu, Pb, Se, As, and Hg in individuals, (3) whole-body ^{137}Cs activity (Bq/g *ww*), (4) migratory status, and (5) month of capture. Site contaminant history was a categorical fixed effect, with each site categorized as CCR, NFP, or reference. The concentrations of Zn, Cu, Pb, Se, As, and Hg in the blood (ppm *dw*) and the activity concentrations of ^{137}Cs (Bq/g) in individual birds were included as fixed effects after each was standardized with a mean of zero and standard deviation of one. We included categorical fixed effects for migratory status (migratory or resident) and month (March, April, May, June, or July) because latent parasite infections are known to intensify at the commencement of breeding season in early spring (Valkiunas, 2004). Host species was included as a random effect to account for species differences in infection dynamics. We evaluated predictors for multicollinearity by evaluating whether the Pearson's correlation coefficient exceeded 0.7 for any pairwise combination.

We fit separate GLMMs containing all predictors to each of the four response variables derived from our analysis of avian blood samples: haemosporidian genus richness, presence of *Plasmodium*, presence of *Haemoproteus,* and presence of *Leucocytozoon.* Haemosporidian genus richness was quantified as an integer representing the number of parasite genera detected in a host and ranged from zero (no infection) to three (triple infection with *Plasmodium, Haemoproteus,* and *Leucocytozoon*) and was modeled using a GLMM with a Poisson distribution and log link. The presence of *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* were each quantified as a binary variable and modeled using a GLMM with a binomial distribution and logit link. For all models, we evaluated the significance of predictors using an alpha value of 0.05.

We used a generalized linear model (GLM) with a binomial distribution and logit link to evaluate whether (1) site contaminant history and (2) season of capture predicted the presence of *Plasmodium* in *Culex* vectors. Site contaminant history was included as a categorical fixed effect indicating whether a site was CCR, NFP, or reference. Due to a lower sample size of *Culex* compared to passerines, we evaluated 'season' instead of 'month' as a fixed effect. The variable 'season' contained two categories: spring (April and May) and summer (June and July).

RESULTS

We collected 334 blood samples from 329 individuals representing 31 passerine species. Five individuals were sampled twice with at least one month between sampling events. Because metal concentrations and parasite infections are dynamic in the blood throughout a single season, these five repeat samples were included as separate observations.

Zinc, Cu, and Hg were detected in all samples. Selenium was detected in 99% of samples (MDL = 0.96 ppm), Pb in 18% of samples (MDL = 0.02 ppm), As in 11% of samples (MDL = 0.04 ppm) and ^{137}Cs in 21% of samples (MDA = 1.56 Bq). Average percent recoveries for the certified reference materials were within the accepted range of 80% to 120% for all elements. Blood Se was significantly higher among birds inhabiting CCR sites (μ 5.3, SE 0.2 ppm *dw*) compared to Ref sites (μ 4.2, SE 0.2 ppm *dw*; $H_{(2)}$ =

17.4, $p < 0.001$). Whole-body activity concentrations of Cs^{137} were higher among birds inhabiting NFP sites (µ 0.02, SE 0.00 Bq/g *ww*) compared to reference sites (0.00, SE 0.00 Bq/g *ww*; $H_{(2)} = 8.4$, $p = 0.01$). The concentrations of other trace elements varied among individuals but were not significantly predicted by site contaminant history (Figure 3.1).

The prevalence of haemosporidian parasites in passerines captured on the Savannah River Site was 68.0%. *Plasmodium* infections were found in 55.1% of hosts, *Haemoproteus* in 29.3% of hosts, and *Leucocytozoon* in 12.9% of hosts. We observed coinfections in 26.3% of hosts, including ten individuals with triple infections.

We did not observe collinearity between predictors. Trace element concentrations within birds were weakly correlated, with the strongest correlations between Se and Hg ($r = 0.38$) followed by Cu and Zn ($r = 0.31$). Due to limitations on blood sample quantity and handling time for the smallest birds, sample sizes were lower for blood Hg ($n = 184$) and ^{137}Cs activity ($n = 158$) than for the other trace elements. Therefore, a set of GLMMs that included blood Hg and ^{137}Cs activity as predictors of coinfection and presence of each parasite genus were fit to a subset of the data.

A higher concentration of Se in the blood was associated with a lower likelihood of *Leucocytozoon* infection ($\beta = -0.77 \pm 0.34$, $p = 0.03$; Supporting Information). The presence of *Leucocytozoon* adhered to an apparent blood Se threshold of 6.7 ppm *dw*, where birds with blood Se values above this threshold were uninfected (Figure 3.2). Arsenic concentrations tended to be higher, though not significantly, in birds infected with *Plasmodium* ($\beta = 0.29 \pm 0.17$, $p = 0.09$). Blood concentrations of Zn, Cu, Pb, or Hg and whole-body activity concentrations of ^{137}Cs were not associated with an altered likelihood of infection by any of the haemospiridian genera. Blood contaminant concentrations and whole-body activity concentrations were not associated with altered haemosporidian genus richness.

At the site level, birds inhabiting CCR sites were more likely to be infected with *Haemoproteus* ($\beta = 1.41 \pm 0.55$, $p = 0.01$) and *Leucocytozoon* ($\beta = 1.27 \pm 0.57$, $p = 0.03$) than birds at reference sites. Site contaminant history did not predict the likelihood of *Plasmodium* infection or the number of coinfections.

Compared to birds captured in July, birds captured in April harbored a greater number of coinfections (β 0.60 SE 0.21, $p = 0.004$) and were significantly more likely to be infected with *Plasmodium* (β 1.09 SE 0.51, $p = 0.03$), *Haemoproteus* (β 1.65 SE 0.72, $p = 0.02$), and *Leucocytozoon* (β 3.13 SE 0.99, $p = 0.002$). Migratory status was not a significant predictor in any model.

The likelihood of infection with each of the three haemosporidian parasite genera differed by host species (Figure 3.3), with the highest between-species variance occurring for *Haemoproteus* (σ 6.86, SD 2.62) followed by *Plasmodium* (σ 2.31, SD 1.52) and *Leucocytozoon* (σ 2.23, SD 1.49). Comparatively, between-species variance in the number of coinfections was low (σ 0.20, SD 0.45).

We captured 195 *Culex* vectors, including 65 individuals at reference sites, 45 at CCR sites, and 85 at NFP sites. Capture rates varied widely between months (Figure 3.4). The overall prevalence of *Plasmodium* in *Culex* was 8.2%. *Culex* vectors captured in June and July were significantly more likely to be infected with *Plasmodium* compared to those captured in April and May (β 2.46 SE 1.09, $p = 0.02$; Supporting Information). There was no significant effect of site contaminant history on *Plasmodium* infection in *Culex* mosquitoes.

DISCUSSION

This study investigated whether waste-derived trace elements altered *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* infection in passerine birds and *Culex* vectors. Passerines inhabiting CCR sites accumulated excess concentrations of Se in their blood compared to those at reference sites, which aligns with prior evidence that Se derived from CCR is bioavailable and transferred to terrestrial wildlife (Bryan et al., 2003, Meyer et al., 2014). We found that higher concentrations of Se in the blood were associated with a lower likelihood of *Leucocytozoon* infection within individuals. This finding contradicts our first hypothesis and provides evidence for the alternative – that sublethal Se exposure decreases parasitism within individuals by fostering unfavorable conditions for some parasites.

Selenium is required in trace amounts by living organisms and plays an essential role in immune function (Hoffman and Berry, 2008). As a component of the antioxidant glutathione peroxidase, Se enables hosts to cope with the inflammatory response and oxidative damage caused by parasite infection (Cantor and Tarino, 1982, Nelson et al., 2016). Experimental studies have demonstrated that dietary Se supplementation increases glutathione peroxidase activity and reduces the severity of disease in protozoan-infected animal models (Cantor and Tarino, 1982, Huang and Yang, 2002). On the SRS, birds receiving higher doses of Se due to trophic contaminant transfer may be more capable of amassing an immune response that clears *Leucocytozoon* infection. We did not observe a comparable relationship between blood Se concentration and presence of *Haemoproteus* or *Plasmodium*, possibly due to the chronic nature of infection with these parasites in passerine hosts. In a long-term field study, Rooyen et al. (2013) suggested that the passerine immune system may be better equipped to defend against *Leucocytozoon* compared to *Plasmodium* and *Haemoproteus* after observing that *Leucocytozoon* in great tits (*Parus major*) had higher turnover across seasons than the other genera.

We did not find evidence in support of our second hypothesis that parasite transmission is disrupted among hosts and vectors inhabiting contaminated sites. In fact, passerines captured in CCR areas were more likely to be infected with *Leucocytozoon* and *Haemoproteus* compared to passerines at reference sites. If the interactions between contaminant exposure and parasite infection are antagonistic within hosts, then individuals inhabiting contaminated landscapes are more likely to tolerate both stressors without mortality, thereby increasing opportunities for parasite transmission throughout the host population (Sánchez et al., 2020). This phenomenon has been observed among hosts affected by intestinal helminths and heavy metals (Sures et al., 2017). For instance, in a population of wild common eiders (*Somateria mollissima*), the removal of parasites via anti-helminthic treatment led to reduced survival among individuals affected by Pb exposure (Morrill et al., 2019). The authors suggested that intestinal helminths, which were nearly 100% prevalent in the host population, moderated the effect of Pb toxicity on host survival by sequestering Pb. Here, we raise the possibility that, through its role in alleviating oxidative stress and infection-induced anemia, Se reduces the virulence of *Haemoproteus* and *Leucocytozoon* infections and improves the longevity of hosts, thereby increasing opportunities for vector transmission and elevating site-level prevalence.

The factors governing haemosporidian co-infections in passerines are still poorly understood. We hypothesized that contaminant exposure would weaken host immune response and allow multiple haemosporidia genera to establish within hosts. Contrary to our predictions, trace element burdens were not associated with greater haemosporidian parasite richness in passerines. We did find strong evidence that haemosporidian richness and the likelihoods of single infection with *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* were highest in the month of April, which coincides with peak breeding season in our population. This reflects a well-studied temporal cycle of avian haemosporidian infection where, during breeding, passerines invest maximal energy in reproduction and less energy in immune function (Astudillo et al., 2013, Knowles et al., 2011). The reduced strength of immune defenses facilitates the relapse of latent parasite infections and allows a greater number of parasite genera to compete for limited resources within the host (Valkiunas, 2004). Our findings suggest that the stress associated with breeding is a stronger driver of infection vulnerability than exposure to the metals, metalloids, and radionuclides derived from waste on the SRS.

A remaining unknown in our system is whether contaminant exposure and/or parasite infection affect passerine breeding success. In the aforementioned study on *S. mollissima,* Morrill et al. (2019) found that blood Pb negatively affected clutch size regardless of the presence of intestinal parasites, despite the latter's compensatory effect on host survival. Breeding birds are known to transfer excess blood Se to their eggs, leading to reduced hatchability at high enough levels (Bryan et al., 2003). The average Se concentrations observed in birds at our CCR sites exceed blood thresholds of concern for reproductive impairment in several species (4.8 ppm *dw*; Ohlendorf and Heinz 2011, Werner et al. 2025). The possibility that excess trace elements can decrease parasitism while also lowering breeding success suggests a complex fitness landscape in contaminated habitats and presents an intriguing direction for future study.

The likelihood of *Plasmodium* infection in *Culex* vectors did not differ between waste and reference sites, but *Culex* were more likely to be infected in June and July compared to April and May. This reflects the expected pattern of the annual infection cycle where the avian breeding season triggers a shift from latent to acute *Plasmodium* infection in birds, which in turn infect emergent *Culex* females seeking a bloodmeal (Valkiunas, 2004). The lack of a site-level difference in *Plasmodium* infection among *Culex* suggests that environmental contamination might not hinder the transmission of *Plasmodium* from hosts to vectors by negatively impacting the latter's fitness in our system. We did not sample throughout the entire *Culex* breeding season and therefore cannot determine whether the observed, temporal increases in *Culex* infection prevalence were followed by a second peak in *Plasmodium* prevalence among hosts. However, the lack of difference in *Plasmodium* infection likelihood between year-round resident birds and migratory birds suggests that transmission of *Plasmodium* from vectors to avian hosts is not disrupted on the SRS landscape. Our findings contradict prior laboratory experiments that have demonstrated reduced survival and vector competency among mosquitos exposed to heavy metals (Barreaux et al., 2016, Neff and Dharmarajan, 2021) and ionizing radiation (Cunningham et al., 2020). However, these experiments were conducted on naïve mosquitos rather than those collected from polluted environments. Other studies have demonstrated increased metallothionein expression and associated trace element tolerance in *Culex* and *Anopheles* exposed to heavy metals over multiple generations (Mireji et al., 2010, Sarkar et al., 2004). Given that environmental contaminant concentrations are spatially heterogenous at our sites, *Culex* populations could also be sustained by patches of minimally polluted breeding habitat. Alternatively, our variable *Culex* capture rates and low observed *Plasmodium* prevalence could have limited our power to detect differences in *Culex* infection across sites. The low prevalence observed in our sample could be driven, in part, by the presence of *Culex territans*, an amphibian feeder that does not vector avian *Plasmodium* (Reinhold et al., 2023). The effects of trace element contamination on mosquito vectors warrant further investigation and would benefit from field studies that employ a greater number of site replicates, higher trapping frequency, longer-term sampling periods, and species-level identification.

Our findings highlight counterintuitive effects of trace element contaminants on host-parasite ecology and raise important questions. First, the association between increased dietary Se and decreased haemosporidian infection in passerines should be investigated further. Experimental manipulation of dietary Se, along with regular measurements of infection status, parasitemia, and oxidative stress biomarkers, could reveal relationships between Se supplementation and finer-scale metrics of disease severity in wild passerines. Cornet et al. (2014) conducted a similar study and found that dietary supplementation led to lower *Plasmodium relictum* parasitemia in domestic canaries (*Serinus canaria*), yet further work is required to identify the roles of specific dietary trace elements in this response. Micronutrient supplementation could have practical implications for improving the resistance of wildlife threatened by disease, although such a strategy could increase host reservoir capacity and would require a greater understanding of fitness effects across ecological and temporal scales. Second, our findings demonstrate that landscapes affected by trace element contamination can support wildlife populations with higher prevalences of vector-transmitted parasites. Trace elements derived from CCR and other waste products can circulate widely among wildlife and have implications for their parasites, including those that pose zoonotic risks to humans. Although a comparatively large body of literature has examined the nuanced dynamics of parasite transmission at the urban-wildland interface, fewer studies have been conducted in habitats that remain structurally intact but are chemically altered by anthropogenic contaminants. Such environments attract a variety of wildlife and represent important areas for future research.

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LITERATURE CITED

Ackerman, J. T., Eagles-Smith, C. A., Herzog, M. P., Hartman, C. A., Peterson, S. H., Evers, D. C., Jackson, A. K., Elliott, J. E., Vander Pol, S. S. & Bryan, C. E. 2016. Avian mercury exposure and toxicological risk across western North America: A synthesis. *Science of The Total Environment,* 568**,** 749-769. https://doi.org/10.1016/j.scitotenv.2016.03.071

Allan, S. A., Bernier, U. R. & Kline, D. L. 2005. Evaluation of oviposition substrates and organic infusions on collection of *Culex* in Florida. *Journal of the American Mosquito Control Association,* 21**,** 268-273. https://doi.org/10.2987/8756-971X(2005)21[268:EOOSAO]2.0.CO;2

Astudillo, V. G., Hernández, S. M., Kistler, W. M., Boone, S. L., Lipp, E. K., Shrestha, S. & Yabsley, M. J. 2013. Spatial, temporal, molecular, and intraspecific differences of haemoparasite infection and relevant selected physiological parameters of wild birds in Georgia, USA. *International Journal for Parasitology: Parasites and Wildlife,* 2**,** 178-189. http://dx.doi.org/10.1016/j.ijppaw.2013.04.005

Atkinson, C. T. & Samuel, M. D. 2010. Avian malaria Plasmodium relictum in native Hawaiian forest birds: Epizootiology and demographic impacts on 'apapane Himatione sanguinea. *Journal of Avian Biology,* 41**,** 357-366.

Ayala, A. J., Yabsley, M. J. & Hernandez, S. M. 2020. A review of pathogen transmission at the backyard chicken-wild bird interface. *Fronteirs in veterinary science,* 7**,** 539925. https://doi.org/10.3389/fvets.2020.539925

Barreaux, A. M. G., Barreaux, P., Thievent, K. & Koella, J. C. 2016. Larval environment influences vector competence of the malaria mosquito *Anopheles gambiae*. *MalariaWorld Journal,* 7**,** 1-6. https://doi.org/10.5281/zenodo.10798340

Becker, D. J., Chumchal, M. M., Bentz, A. B., Platt, S. G., Czirják, G. A., Rainwater, T. R., Altizer, S. & Streicker, D. G. 2017. Predictors and immunological correlates of sublethal mercury exposure in vampire bats. *Royal Society Open Science,* 4**,** 170073. http://dx.doi.org/10.1098/rsos.170073

Becker, D. J., Chumchal, M. M., Broders, H. G., Korstian, J. M., Clare, E. L., Rainwater, T. R., Platt, S. G., Simmons, N. B. & Fenton, M. B. 2018. Mercury bioaccumulation in bats reflects dietary connectivity to aquatic food webs. *Environmental Pollution,* 233**,** 1076-1085. https://doi.org/10.1016/j.envpol.2017.10.010

Bowerman, W. W., Giesy, J. P., Best, D. A. & Kramer, V. J. 1995. A review of factors affecting productivity of bald eagles in the Great Lakes region: implications for recovery. *Environmental Health Perspectives,* 103**,** 51-59. https://doi.org/10.1289/ehp.95103s451

Bryan, A. L., Jr., Hopkins, W. A., Baionno, J. A. & Jackson, B. P. 2003. Maternal transfer of contaminants to eggs in common grackles (*Quiscalus quiscala*) nesting on coal fly ash basins. *Archives of Environmental Contamination and Toxicology,* 45**,** 273-277. https://doi.org/10.1007/s00244-002-0212-9

Burbacher, T. M., Sackett, G. P. & Mottet, N. K. 1990. Methylmercury effects on the social behavior of *Macaca fascicularis* infants. *Neurotoxicology and Teratology,* 12**,** 65-71. https://doi.org/10.1016/0892-0362(90)90114-R

Burger, J. 1993. Metals in avian feathers: bioindicators of environmental pollution. *Reviews of Environmental Contamination and Toxicology***,** 203-311.

Burkett-Cadena, N. 2013. *Mosquitoes of the Southeastern United States*, University of Alabama Press.

Cable, A. B., Willcox, E. V. & Leppanen, C. 2022. Contaminant exposure as an additional stressor to bats affected by white-nose syndrome: Current evidence and knowledge gaps. *Ecotoxicology,* 31**,** 12-23. https://doi.org/10.1007/s10646-021-02475-6

Cantor, A. H. & Tarino, J. Z. 1982. Comparative effects of inorganic and organic dietary sources of selenium on selenium levels and selenium-dependent glutathione peroxidase activity in blood of young turkeys. *The Journal of Nutrition,* 112**,** 2187-2196. https://doi.org/10.1093/jn/112.11.2187

Carlton, W. H., Bauer, L. R., Evans, A. G., Geary, L. A., Murphy Jr., C. E., Pinder, J. E. & Strom, R. N. 1992. Cesium in the Savannah River Site environment. U.S. Department of Energy.

Cornet, S., Bichet, C., Larcombe, S., Faivre, B. & Sorci, G. 2014. Impact of host nutritional status on infection dynamics and parasite virulence in a bird-malaria system. *Journal of Animal Ecology,* 83**,** 256-265. https://doi.org/10.1111/1365-2656.12113

Cunningham, C. A., Aldridge, R. L., Kline, J., Bibbs, C. S., Linthicum, K. J. & Xue, R. 2020. Effects of radiation on blood-feeding activity of Aedes aegypti (Diptera: Culicidae). *Journal of Vector Ecology,* 45**,** 135-136. https://doi.org/10.1111/jvec.12382

Currie, L. A. 1968. Limits for qualitative detection and quantitative determination. *Analytical Chemistry,* 40**,** 586-593. https://doi.org/10.1021/ac60259a007

Daszak, P., Cunningham, A. A. & Hyatt, A. D. 2000. Emerging infectious diseases of wildlife-- threats to biodiversity and human health. *Science,* 287**,** 443-449. https://doi.org/10.1126/science.287.5452.443

Deonarine, A., Schwartz, G. E. & Ruhl, L. S. 2023. Environmental impacts of coal combustion residuals: Current understanding and future perspectives. *Environmental Science and Technology,* 57**,** 1855-1869. https://doi.org/10.1021/acs.est.2c06094

Dunn, R. R., Harris, N. C., Colwell, R. K., Koh, L. P. & Sodhi, N. S. 2009. The sixth mass coextinction: are most endangered species parasites and mutualists? *Proceedings of the Royal Society B,* 276**,** 3037-3045. https://doi.org/10.1098/rspb.2009.0413

Dwivedi, D., Steefel, C. I., Arora, B., Banfield, J., Bargar, J., Boyanov, M. I., Brooks, S. C., Chen, X., Hubbard, S. S., Kaplan, D., Kemner, K. M., Nico, P. S., O'Loughlin, E. J., Pierce, E. M., Painter, S. L., Scheibe, T. D., Wainwright, H. M., Williams, K. H. & Zavarin, M. 2022. From legacy contamination to watershed systems science: a review of scientific insights and technologies developed through DOE-supported research in water and energy security. *Environmental Research,* 17. https://doi.org/10.1088/1748-9326/ac59a9

Ellis, V. A., Huang, X., Westerdahl, H., Jönsson, J., Hasselquist, D., Neto, J. M., Nilsson, J., Hegemann, A., Hellgren, O. & Bensch, S. 2020. Explaining prevalence, diversity and host specificity in a community of avian haemosporidian parasites. *Oikos,* 129**,** 1314-1329. https://doi.org/10.1111/oik.07280

Estrada, J. M. & Bhamidimarri, R. 2016. A review of the issues and treatment options for wastewater from shale gas extraction by hydraulic fracturing. *Fuel,* 182**,** 292-303. https://doi.org/10.1016/j.fuel.2016.05.051

Ezenwa, V. O., Godsey, M. S., King, R. J. & Guptill, S. C. 2006. Avian diversity and West Nile virus: testing associations between biodiversity and infectious disease risk. *Proceedings of the Royal Society B,* 273**,** 109-117. 10.1098/rspb.2005.3284

Finkelstein, M. E., Doak, D. F., Georgia, D., Burnett, J., Brandt, J. E., Church, M., Grantham, J. & Smith, D. R. 2012. Lead poisoning and the deceptive recovery of the critically endangered California condor. *Proceedings of the National Academy of Sciences,* 109**,** 11449-11454. www.pnas.org/cgi/doi/10.1073/pnas.1203141109

Foley, D. H., Harrison, G., Murphy, J. R., Dowler, M., Rueda, L. M. & Wilkerson, R. C. 2012. Mosquito bisection as a variable in estimates of PCR-derived malaria sporozoite rates. *Malaria Journal,* 11.

Hellgren, O., Waldenstrom, J. & Bensch, S. 2004. A new PCR assay for simultaneous studies of Leucocytozoon, Plasmodium, and Haemoproteus from avian blood. *Journal of Parasitology,* 90**,** 797-802. https://doi.org/10.1645/GE-184R1

Hoffman, P. R. & Berry, M. J. 2008. The influence of selenium on immune responses. *Molecular Nutrition & Food Research,* 52**,** 1273-1280. https://doi.org/10.1002/mnfr.200700330

Hoyt, J. R., Kilpatrick, A. M. & Langwig, K. E. 2021. Ecology and impacts of white-nose syndrome on bats. *Nature Reviews Microbiology,* 19**,** 196-210. https://doi.org/10.1038/s41579-020-00493-5

Huang, K. & Yang, S. 2002. Inhibitory effect of selenium on *Cryptosporidium parvum* infection in vitro and in vivo. *Biological Trace Element Research,* 90**,** 261-272. https://doi.org/10.1385/BTER:90:1-3:261

Kapustka, L. A. 2004. Issue paper on the ecological effects of metals.: US Environmental Protection Agency.

Keesing, F. & Ostfeld, R. S. 2021. Impacts of biodiversity and biodiversity loss on zoonotic diseases. *PNAS,* 118**,** e2023540118. https://doi.org/10.1073/pnas.2023540118

Kesäniemi, J., Jernfors, T., Lavrinienko, A., Kivisaari, K., Kiljunen, M., Mappes, T. & Watts, P. C. 2019. Exposure to environmental radionuclides is associated with altered metabolic and immunity pathways in a wild rodent. *Molecular Ecology,* 28**,** 4620-4635. https://doi.org/10.1111/mec.15241

Kiesecker, J. M. 2002. Synergism between trematode infection and pesticide exposure: A link to amphibian limb deformities in nature? *PNAS,* 99**,** 9900-9904. www.pnas.org/cgi/doi/10.1073/pnas.152098899

Knowles, S. C., Wood, M. J., Alves, R., Wilkin, T. A., Bensch, S. & Sheldon, B. C. 2011. Molecular epidemiology of malaria prevalence and parasitaemia in a wild bird population. *Molecular Ecology,* 20.

Kojima, L. V., Kohl, M. T., Rainwater, T. R., Parrot, B. B. & Tuberville, T. D. 2024. Association of size, climatic factors, and mercury body burdens with movement behavior in American alligators. *Science of The Total Environment,* 921**,** 170859. https://doi.org/10.1016/j.scitotenv.2024.170859

Koller, L. D. 1980. Immunotoxicology of heavy metals. *International Journal of Immunopharmacology,* 2**,** 269-270.

Kramer, V. J., Etterson, M. A., Hecker, M., Murphy, C. A., Roesijadi, G., Spade, D. J., Spromberg, J. A., Wang, M. & Ankley, G. T. 2010. Adverse outcome pathways and ecological risk assessment: Bridging to population-level effects. *Environmental Toxicology and Chemistry,* 30**,** 64-76. https://doi.org/10.1002/etc.375

Lafferty, K. D. & Kuris, A. M. 1999. How environmental stress affects the impacts of parasites. *Limnology and Oceanography,* 44**,** 925-931. https://doi.org/10.4319/lo.1999.44.3_part_2.0925

Lotta, I. A., Valkiūnas, G., Pacheco, M. A., Escalante, A. A., Hernández, S. R. & Matta, N. E. 2019. Disentangling *Leucocytozoon* parasite diversity in the neotropics: descriptions of two new species and shortcomings of molecular diagnostics for leucocytozoids. *International Journal for Parasitology: Parasites and Wildlife,* 9**,** 159-173. https://doi.org/10.1016/j.ijppaw.2019.05.002

Maklakov, A. A., Immler, S., Gonzalez-Voyer, A., Rönn, J. & Kolm, N. 2011. Brains and the city: big-brained passerine birds succeed in urban environments. *Biology Letters,* 7**,** 730-732. https://doi.org/10.1098/rsbl.2011.0341

Marcogliese, D. J. 2005. Parasites of the superorganism: Are they indicators of ecosystem health? *International Journal for Parasitology,* 35**,** 705-716. https://www.doi.org/10.1016/j.ijpara.2005.01.015

Marcogliese, D. J. & Pietrock, M. 2011. Combined effects of parasites and contaminants on animal health: parasites do matter. *Trends in Parasitology,* 27**,** 123-130. https://www.doi.org/10.1016/j.pt.2010.11.002

Meyer, C. B., Schlekat, T. H., Walls, S. J., Iannuzzi, J. & Souza, M. J. 2014. Evaluating risks to wildlife from coal fly ash incorporating recent advances in metals and metalloids risk assessment.

Integrated Environment Assessment and Management, 11**,** 67-79. https://doi.org/10.1002/ieam.1576

Miller, R. S., Farnsworth, M. L. & Malmberg, J. L. 2013. Diseases at the livestock-wildlife interface: Status, challenges, and opportunities in the United States. *Preventative Veterinary Medicine,* 110**,** 119-132. https://doi.org/10.1016/j.prevetmed.2012.11.021

Mireji, P. O., Keating, J., Hassanali, A., Impoinvil, D. E., Mbogo, C. M., Muturi, M. N., Nyambaka, H., Kenya, E. U., Githure, J. I. & Beier, J. C. 2010. Expression of metallothionein and α-tubulin in heavy metal-tolerant *Anopheles gambiae* sensu stricto (Diptera: Culicidae). *Ecotoxicology and Environmental Safety,* 73**,** 46-50. https://doi.org/10.1016/j.ecoenv.2009.08.004

Morales-Castilla, I., Pappalardo, P., Farrell, M. J., Aguirre, A. A., Huang, S., Gehman, A. M., Dallas, T., Gravel, D. & Davies, T. J. 2021. Forecasting parasite sharing under climate change. *Philosophical Transactions of the Royal Society B,* 376**,** 20200360. https://doi.org/10.1098/rstb.2020.0360

Morley, N. J., Lewis, J. W. & Hoole, D. 2006. Pollutant-induced effects on immunological and physiological interactions in aquatic host-trematode systems: implications for parasite transmission. *Journal of Helminthology,* 80**,** 137-149. https://www.doi.org/10.1079/JOH2006345

Morrill, A., Provencher, J. F., Gilchrist, H. G., Mallory, M. L. & Forbes, M. R. 2019. Anti-parasite treatment results in decreased estimated survival with increasing lead (Pb) levels in the common eider *Somateria mollissima*. *Proceedings of the Royal Society B,* 286**,** 20191356. https://doi.org/10.1098/rspb.2019.1356

Neff, E. & Dharmarajan, G. 2021. The direct and indirect effects of copper on vector-borne disease dynamics. *Environmental Pollution,* 269. https://doi.org/10.1016/j.envpol.2020.116213

Nelson, S. M., Shay, A. E., James, J. L., Carlson, B. A., Urban, J. F. & Prabju, K. S. 2016. Selenoprotein expression in macrophages is critical for optimal clearance of parasitic helminth *Nippostrongylus brasiliensis*. *Journal of Biological Chemistry,* 291**,** 2787-2798. https://doi.org/10.1074/jbc.M115.684738

O'Meara, G. F., Vose, F. E. & Carlson, D. B. 1989. Environmental factors influencing oviposition by *Culex* (Diptera: Culcidae) in two types of traps. *Journal of Medical Entomology,* 26**,** 528-534.

Ohlendorf, H. M. & Heinz, G. H. 2011. Selenium in birds. *In:* Beyer, W. N. & Meador, J. P. (eds.) *Environmental Contaminants in Biota: Interpreting Tissue Concentrations.* 2 ed. Boca Raton, FL, USA: CRC.

Oldenkamp, R. E., Bryan Jr., A. L., Kennamer, R. A., Leaphart, J. C., Webster, S. C. & Beasley, J. C. 2017. Trace elements and radiocesium in game species near contaminated sites. *The Journal of Wildlife Management,* 81**,** 1338-1350. https://doi.org/10.1002/jwmg.21314

Patz, J. A., Graczyk, T. K., Geller, N. & Vittor, A. Y. 2000. Effects of environmental change on emerging parasitic diseases. *International Journal for Parasitology,* 30. https://doi.org/10.1016/S0020-7519(00)00141-7

Pigeault, R., Cozzarolo, C. S., Choquet, R., Strehler, M., Jenkins, T., Delhaye, J., Bovet, L., Wassef, J., Glaizot, O. & Christe, P. 2018. Haemosporidian infection and co-infection affect host survival and reproduction in wild populations of great tits. *International Journal for Parasitology,* 48**,** 1079-1087. https://doi.org/10.1016/j.ijpara.2018.06.007

Poulin, R. 1992. Toxic pollution and parasitism in freshwater fish. *Parasitology Today,* 8**,** 58-61.

Reinhold, J. M., Halbert, E., Roark, M., Smith, S. N., Stroh, K. M., Siler, C. D., McLeod, D. S. & Lahondère, C. 2023. The role of *Culex territans* mosquitoes in the transmission of *Batrachochytrium dendrobatidis* to amphibian hosts. *Parasites & Vectors,* 16**,** 424. https://doi.org/10.1186/s13071-023-05992-x

Riley, S., Serieys, L. & Moriarty, J. 2014. Infectious diseases and contaminants in urban wildlife: unseen and often overlooked threats. *In:* McCleery, R., Moorman, C. & Peterson, M. (eds.) *Urban Wildlife Conservation.* Boston, MA, USA: Springer.

Rooyen, J. v., Lalubin, F., Glaizot, O. & Christe, P. 2013. Avian haemosporidian persistence and co-infection in great tits at the individual level. *Malaria Journal,* 12. https://doi.org/10.1186/1475-2875-12-40

Saaristo, M., Brodin, T., Balshine, S., Bertram, M. G., Brooks, B. W., Ehlman, S. M., McCallum, E. S., Sih, A., Sundin, J., Wong, B. B. M. & Arnold, K. E. 2018. Direct and indirect effects of chemical contaminants on the behaviour, ecology and evolution of wildlife. *Proceedings of the Royal Society B,* 285. http://dx.doi.org/10.1098/rspb.2018.1297

Sánchez, C. A., Altizer, S. & Hall, R. 2020. Landscape-level toxicant exposure mediates infection impacts on wildlife populations. *Biology Letters,* 16**,** 20200559. http://dx.doi.org/10.1098/rsbl.2020.0559

Sarkar, S., Duttagupta, A. K. & Mal, T. K. 2004. Effects of heavy metals on population growth and metallothionein gene expression in the mosquito *Culex quinquefasciatus*, from Calcutta, India. *Environmental Pollution,* 127**,** 183-193. https://www.doi.org/10.1016/j.envpol.2003.08.005

Seewagen, C. L. & Newhouse, M. 2018. Mass changes and energetic condition of grassland and shrubland songbirds during autumn stopovers at a reclaimed landfill in the New Jersey meadowlands. *The Wilson Journal of Ornithology,* 130**,** 377-384. https://doi.org/10.1080/00063657.2021.1960269

SRNS. 2020. Comprehensive alternative evaluation of ash and coal fine waste units at the Savannah River Site.

SRNS. 2022. Savannah River Site Environmental Report.

Sternalski, A., Matsui, S., Bonzom, J. M., Kasahara, S., Beaugelin-Seiller, K., Ueda, K., Watanabe, M. & Adam-Guillermin, C. 2015. Assessment of radiological dose rates in passerine birds after the Fukushima nuclear accident according to their life stage. *Japanese Journal of Ornithology,* 64**,** 161-168. https://doi.org/10.3838/jjo.64.161

Sures, B., Nachev, M., Selbach, C. & Marcogliese, D. J. 2017. Parasite responses to pollution: what we know and where we go in 'Environmental Parasitology'. 10. https://doi.org/10.1186/s13071-017-2001-3

UNEP. 2020. An Assessment Report on Issues of Concern: Chemicals and Waste Issues Posing Risks to Human Health and the Environment.

Valkiunas, G. 2004. *Avian Malaria Parasites and Other Haemosporidia,* Boca Raton, FL, USA, CRC Press.

Werner, C. S., Chapman, M., Rhodes Jr., O. E. & DeVault, T. L. 2025. Uptake of metals, metalloids, and radiocesium varies with habitat use among passerine communities at coal combustion and nuclear fission legacy waste sites. *Environmental Pollution,* 367**,** 125608. https://doi.org/10.1016/j.envpol.2024.125608

Werner, C. S., Chapman, M., Skaggs, J., Rhodes Jr., O. E. & DeVault, T. L. 2024. Trace metal transfer to passerines inhabiting wastewater treatment wetlands. *Science of The Total Environment,* 927**,** 172373. https://doi.org/10.1016/j.scitotenv.2024.172373

Werner, C. S. & Nunn, C. L. 2020. Effect of urban habitat use on parasitism in mammals: a meta-analysis. *Proceedings of the Royal Society B,* 287**,** 20200397. https://doi.org/10.1098/rspb.2020.0397

White, D. L. & Gaines, K. F. 2000. The Savannah River Site: Site description, land use and management history. *Avian Research at the Savannah River Site: A Model for Integrating Basic Research and Long-Term Management.* Scholarly Commons.

Williams, G. M. & Gingrich, J. B. 2007. Comparison of light traps, gravid traps, and resting boxes for West Nile virus surveillance. *Journal of Vector Ecology,* 32**,** 285-291. https://doi.org/10.3376/1081-1710(2007)32[285:COLTGT]2.0.CO;2

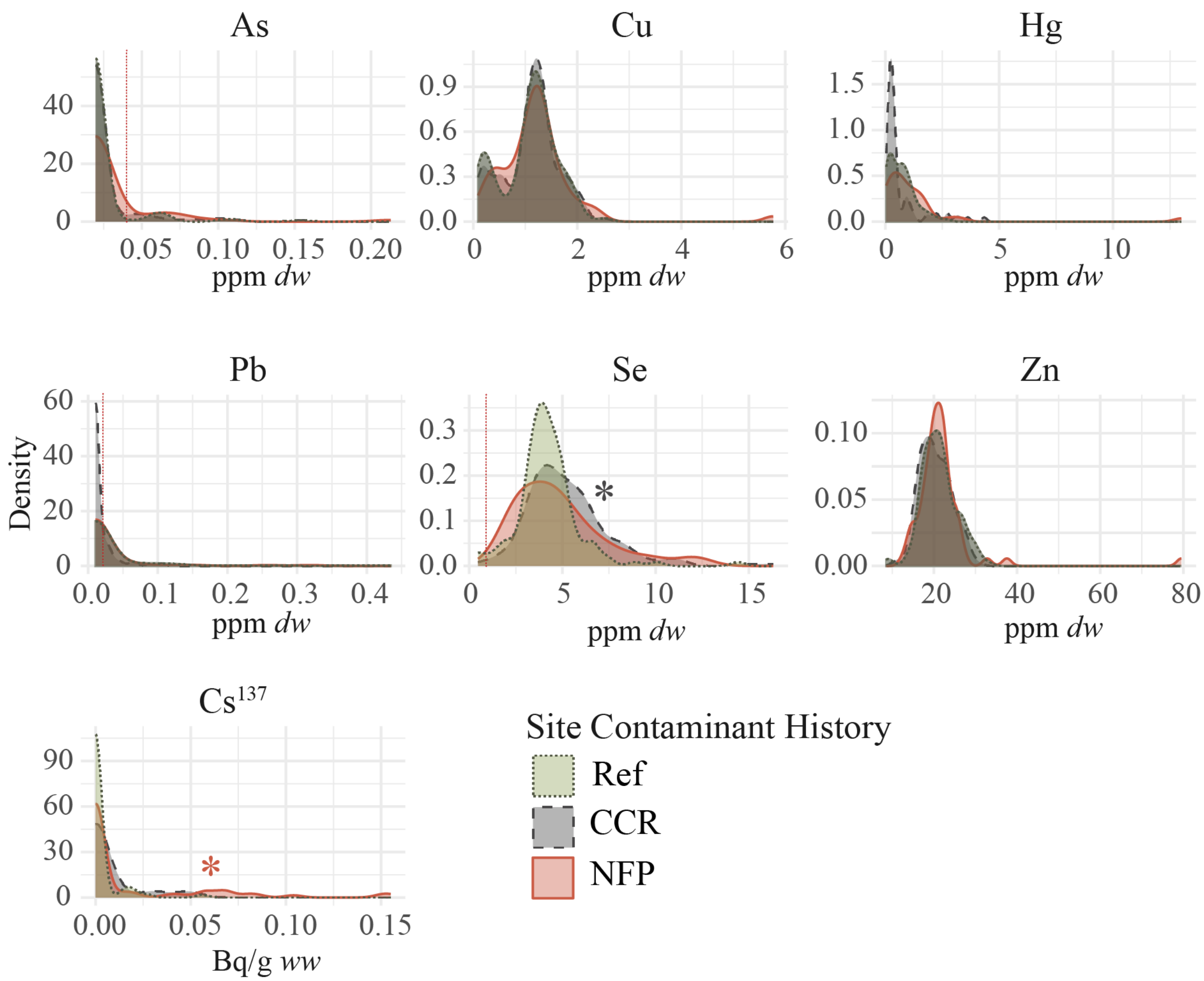

Figure 3.1. Density plots showing the distribution of As, Cu, Hg, Pb, Se, and Zn concentrations (ppm dry weight) in the blood, and ^{137}Cs activity (Bq/g wet weight) in the whole body of passerine birds inhabiting polluted areas affected by nuclear fission products (NFP) or coal combustion residuals (CCR) and reference areas (Ref) on the Savannah River Site. Red, vertical lines indicate method detection limits. Asterisks indicate whether an element was significantly elevated at CCR or NFP sites compared to Reference sites, as indicated by Kruskal-Wallis tests ($\alpha = 0.05$).

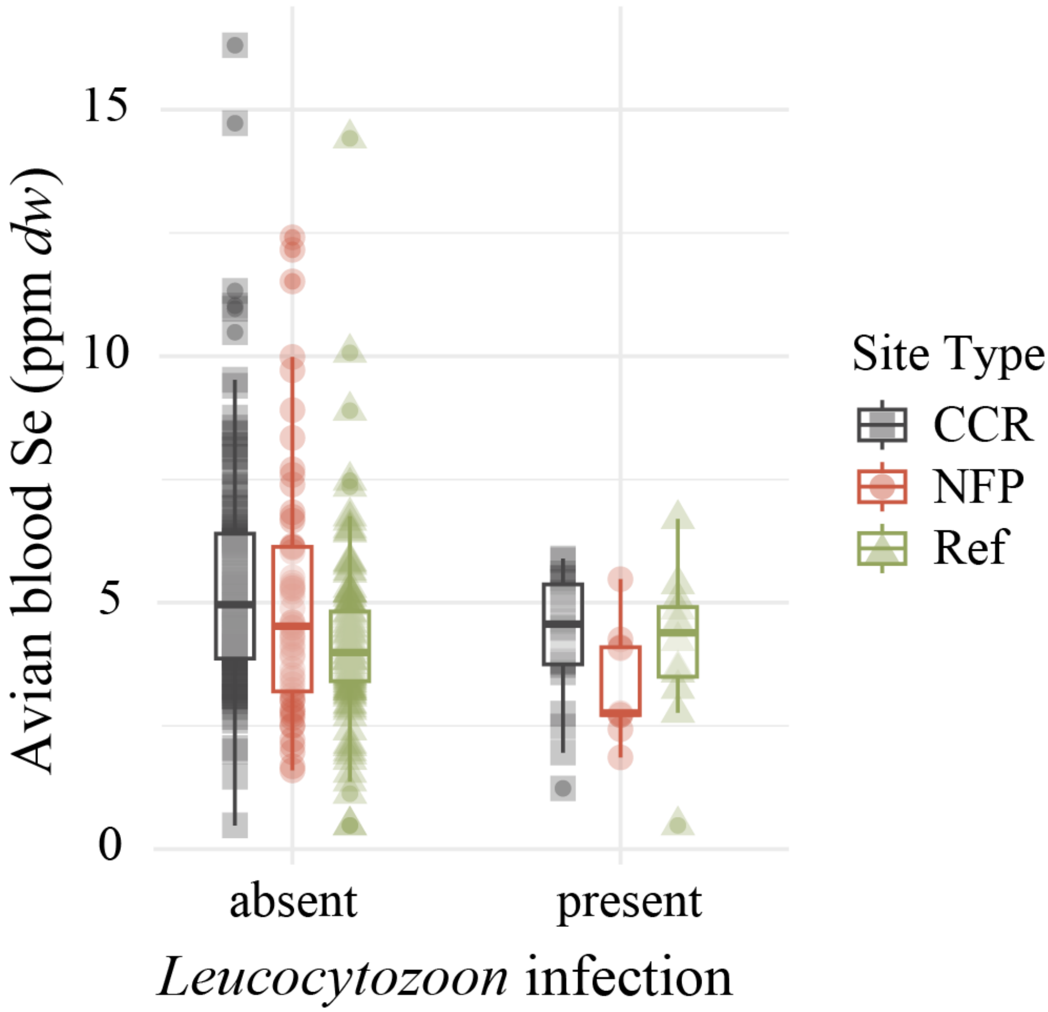

Figure 3.2. Blood Se concentrations (ppm *dw*) in birds with and without *Leucocytozoon* infection.

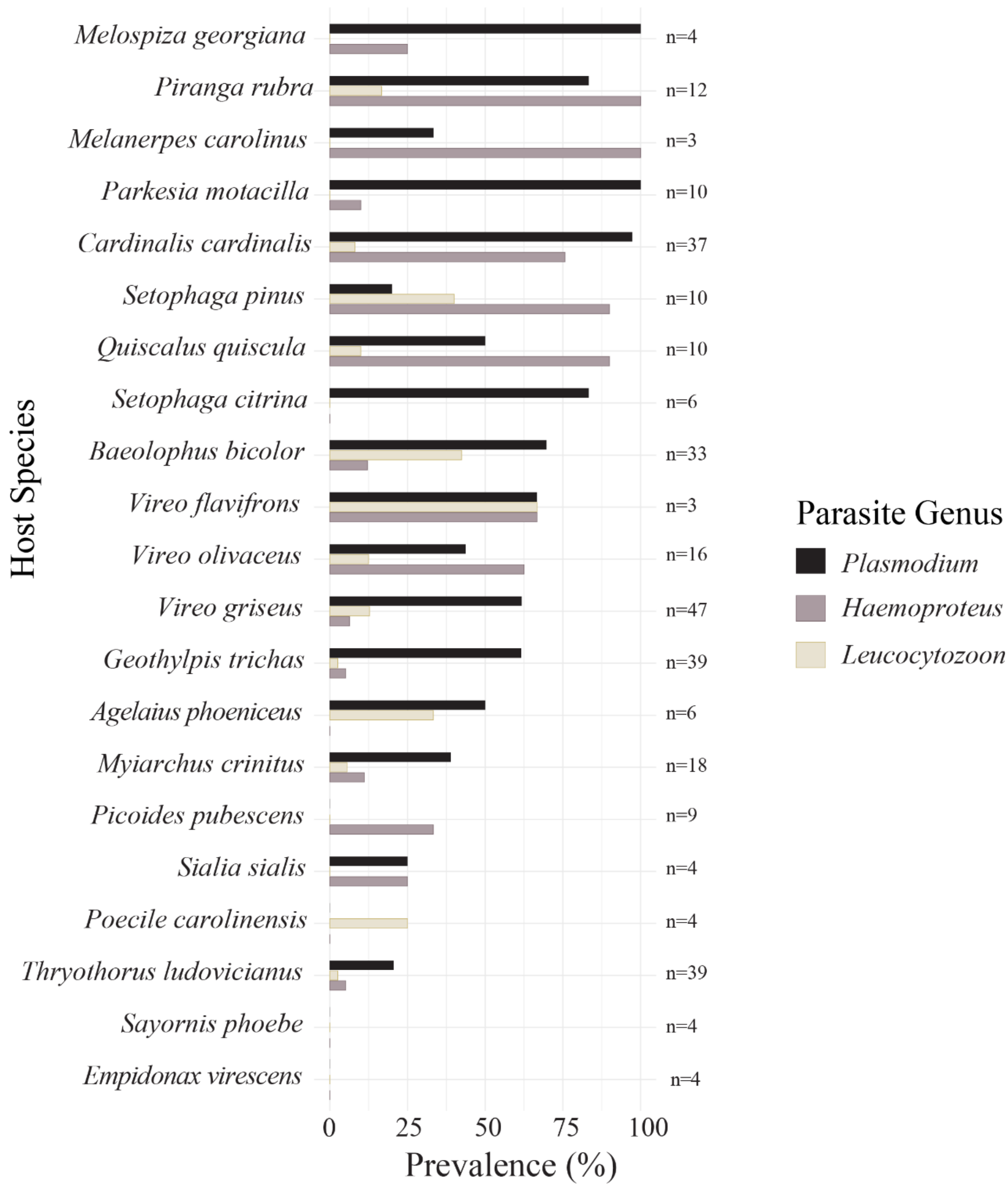

Figure 3.3. The prevalence of haemosporidian parasites in passerine host species on the Savannah River Site, excluding species for which n < 3.

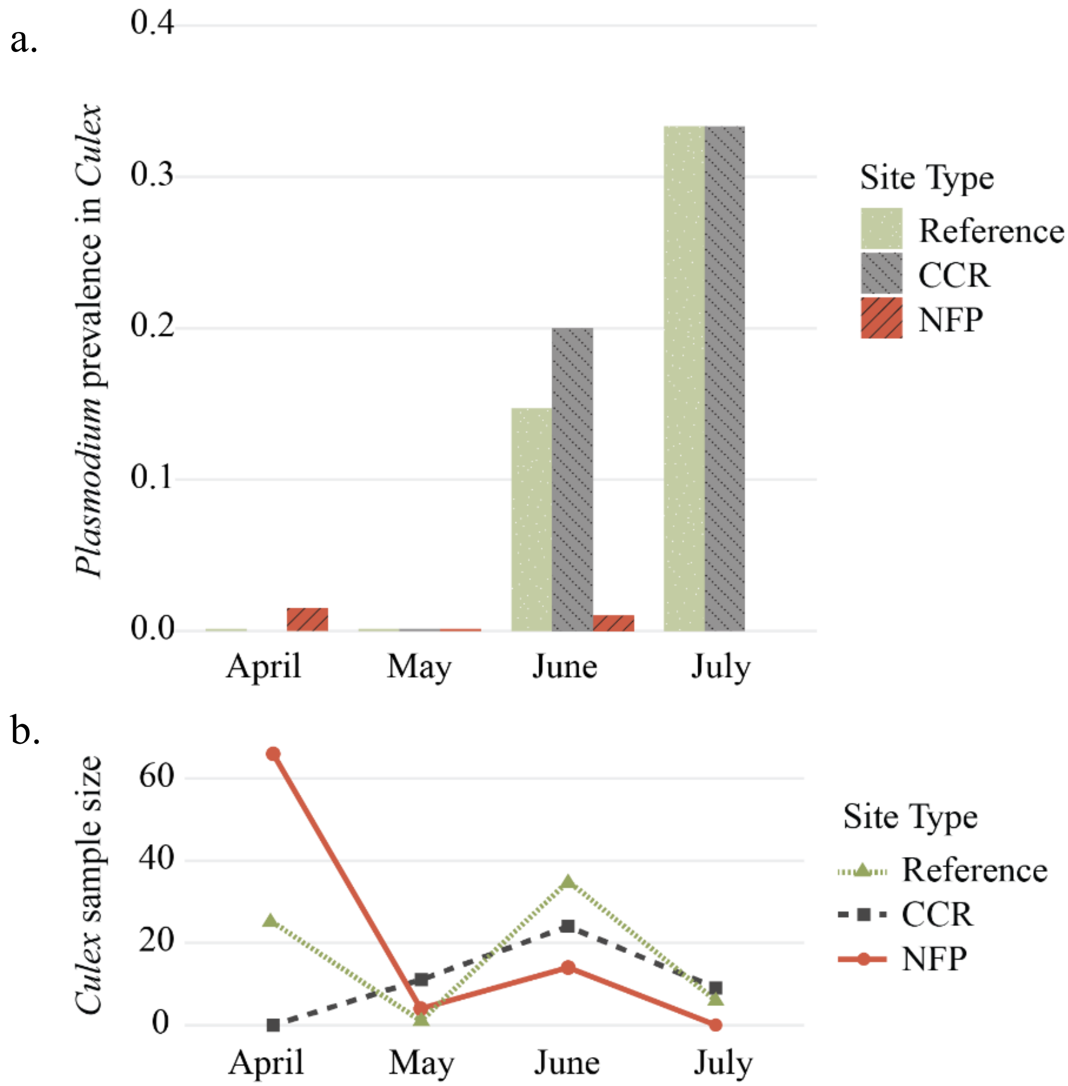

Figure 3.4.a. Prevalence of *Plasmodium* in *Culex* mosquitoes sampled from April through July at sites affected by coal combustion residuals or nuclear fission products and reference sites. **b.** Number of *Culex* captured per sampling event.

CHAPTER 4

SOUNDSCAPE INDICES REVEAL DISRUPTED BIOACOUSTIC ACTIVITY IN RIPARIAN ECOSYSTEMS AFFECTED BY LEGACY HAZARDOUS WASTES

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ABSTRACT

Freshwater pollution has far-reaching impacts on the biotic communities of aquatic and riparian ecosystems. Biological monitoring initiatives in contaminated habitat often rely on small subsets of indicator species, such as aquatic macroinvertebrates, and would benefit from additional approaches to measuring ecological integrity at larger scales. Passive acoustic technologies allow for efficient, long-term monitoring of the communities that rely on riparian habitat, but their utility in assessing contaminant impacts on ecosystems remains unexplored. This study investigated the use of acoustic indices to detect ecological degradation due to chemical disturbance from radiological and non-radiological hazardous wastes on the Savannah River Site. Passive acoustic recorders sampled the dawn and dusk soundscapes at nineteen riparian sites from 28 June to August 02, 2024, and the bioacoustic index, acoustic complexity index, acoustic diversity index, and event count index were calculated for each minute of audio. Historical data on aquatic macroinvertebrate and fish communities were collected from the same sites. Linear mixed effects models were used to evaluate whether the acoustic indices varied with hazardous waste area, cesium-137 dose rates, and habitat type and structure in the vicinity of each detector. We found that the relative amplitude of the dawn chorus, as measured by the bioacoustic index, was significantly lower at sites affected by greater areas of hazardous waste, and this pattern reflected trends in the aquatic macroinvertebrate index across sites. Conversely, the dusk bioacoustic index was significantly higher at hazardous waste sites. None of the acoustic indices varied with cesium-137 dose rates, but sound evenness and temporal complexity varied with area of wetland cover and forest structure. Our findings demonstrate that soundscapes are sensitive indicators of riparian ecosystem function in contaminated environments, and passive acoustic monitoring may be a cost-effective means of monitoring responses to hazardous waste influx and remediation initiatives.

INTRODUCTION

The improper management of industrial waste leads to influxes of potentially toxic elements into freshwater systems (Dwivedi et al., 2022, United Nations, 2024) . Waste products from the extraction and use of fossil fuels and mineral resources, including coal, natural gas, and uranium, contaminate freshwater resources on every continent (Bargagli, 2008, Ferrari et al., 2017, Rozell and Reaven, 2011, Walters et al., 2011, Zhou et al., 2020). The accumulation of excess metals, metalloids, and radionuclides in freshwater threatens public and environmental health by limiting the accessibility of safe drinking water and degrading aquatic habitat (Mason, 2002, Yang et al., 2024). Freshwater pollution also affects semi-aquatic or terrestrial biota inhabiting riparian zones, either directly through the trophic transfer of contaminants (Becker et al., 2018, Werner et al., 2025), or indirectly due to the cascading consequences of aquatic biodiversity loss (Mattsson and Cooper, 2006).

The remediation of polluted freshwater environments requires an understanding of contaminant impacts on aquatic and riparian ecosystems. Traditional evaluations of aquatic habitat often rely on the use of biological indicators, or organisms whose presence or absence provide an indication of environmental quality (Clark et al., 2021, Phillips and Rainbow, 1994). Surveys of multiple biological indicator species provide the basis for community-scale metrics of ecological integrity. For example, the diversity and abundance of mayflies (Ephemeroptera), stoneflies (Plecoptera), and caddisflies (Trichoptera), are widely used to derive biotic indices of stream quality due to the sensitivities of these taxa to aquatic pollution (Rosenberg and Resh, 1993). Surveys of amphibians and riparian birds complement those of aquatic macroinvertebrates by providing insight into ecosystem function within the riparian corridor (Mattsson and Cooper, 2006, Stapanian et al., 2015). However, in practice, traditional surveys of biological indicators are time- and resource-intensive, which often limits their scope, and, consequently, their power to detect contaminant impacts on ecosystems (Gewurtz et al., 2011). Biomonitoring initiatives on polluted lands would benefit from innovative, noninvasive, and repeatable approaches to measuring ecological integrity at larger spatial, temporal, and taxonomic scales (Rhodes et al., 2020).

Recent developments in passive acoustic technology allow for efficient, long-term monitoring of soundscapes, defined as the collection of sounds derived from the biological, physical, and anthropogenic components of a landscape (Pijanowski et al., 2011, Pijanowski, 2024). The distribution of acoustic energy across frequencies and over time contains a wealth of information on the diversity, abundance, and health of acoustically active organisms at a given site. A growing number of acoustic indices have been developed to aid in the interpretation of soundscapes and their relationship to biodiversity (Boelman et al., 2007, Bradfer-Lawrence et al., 2024, Gasc et al., 2013). Recent studies have demonstrated that trends in acoustic indices reflect ecological responses to a variety of disturbances, including fire (Gasc et al., 2013), logging (Burivalova et al., 2019), and noise pollution (Grinfeder et al., 2022).

Acoustic indices have the potential to reveal ecosystem-scale responses to contamination, though their utility in this context remains unexplored. The communities that contribute most to the soundscapes of riparian ecosystems, including insects, anurans, and passerines, are thought to exhibit lower abundance and/or species richness in habitat contaminated with waste-derived radionuclides (Moller and Mousseau, 2007) and trace elements (Sievers et al., 2018, Smalling et al., 2019). Additionally, exposure to heavy metals can affect vocal performance in vertebrates by impairing the development of required neural pathways or vocal apparatuses (Duan and Huang, 2016, Goodchild et al., 2021) or by reducing overall health (Gorissen et al., 2005). Given the potential for adverse effects of waste-derived contaminants on acoustically active organisms, their presence in freshwater environments should lead to altered riparian soundscapes that are detectable via passive acoustic monitoring.

The aim of this study was to investigate the use of four acoustic indices—the bioacoustic index (BI), acoustic complexity index (ACI), acoustic diversity index (ADI), and event count index (EVN)—to detect changes in riparian ecosystem function due to chemical disturbance from coal combustion and nuclear fission legacy wastes. We also investigated whether trends in acoustic indices derived from riparian soundscapes reflect those of traditional, biotic indices derived from aquatic macroinvertebrate and fish surveys. We hypothesized that contaminated, riparian habitats are characterized by communities of acoustically active organisms that have lower abundance, diversity, and/or health compared to those in uncontaminated, riparian habitat. Therefore, we predicted that the dawn and dusk soundscapes of contaminated habitats would be characterized by lower values of (1) the BI, indicating a lower intensity of acoustic activity, (2) the ACI, indicating lower complexity of sound across time, (3) the ADI, indicating reduced evenness of sound across frequency bands, and (4) the EVN, indicating fewer discrete acoustic events. We also hypothesized that riparian soundscape integrity is linked to aquatic habitat quality and thus expected to observe comparable trends in both acoustic indices and traditional, biotic indices across habitats with varying waste impacts.

METHODS

Site Background

The Savannah River Site (SRS) is a U.S. Department of Energy property in the Sandhills region of South Carolina that encompasses five tributaries of the Savannah River (White and Gaines, 2000). From the 1950s until the 1980s, the SRS operated nine coal-fired power plants and five nuclear reactors to produce fissile material for the fabrication of nuclear weapons. Freshwater systems located upstream of industrial areas were minimally impacted by contamination from SRS operations, but those located downstream received discharges of nuclear fission products and coal combustion residuals that contained potentially toxic metals, metalloids, and radionuclides (Carlton et al., 1992, SRNS, 2020).

In the 1990s, the U.S. Department of Energy (US DOE), the U.S. Environmental Protection Agency (US EPA), and the South Carolina Department of Environmental Services (SCDES) prioritized the SRS for remediation (USEPA, 1996). Due to the expense of excavating contaminated media, the ongoing management strategy relies on administrative controls that leave hazardous waste in place while limiting disturbance. However, hazardous waste areas are not isolated from surrounding ecosystems, and contaminants continue to cycle among biotic and abiotic compartments (Fletcher et al., 2014, Fulghum et al., 2019, Leaphart et al., 2019, Werner et al., 2025). To monitor ongoing contaminant impacts on freshwater systems, the US DOE conducts periodic surveys of the aquatic macroinvertebrate and fish communities at designated sites across the drainage areas of the five major tributaries (Table 1; Figure 4.1). The Multiple Habitat Sampling Protocol (MHSP) was developed to evaluate the integrity of aquatic macroinvertebrate communities in South Carolina coastal plain streams and has been tailored for use on the SRS (Paller et al., 2007). The MHSP index is derived from the diversity and abundance of Ephemeroptera, Plecoptera, and Trichoptera taxa that have variable sensitivities to aquatic pollution and thus serve as bioindicators of stream integrity (Paller et al., 2007, SCDES, 1998). The Index of Biotic Integrity (IBI) is calculated from fish assemblage data and has also been adapted for use on the SRS (Paller et al., 1996). Long term trends in the MHSP and IBI reveal disparities in the integrity of SRS streams over a 30-year period (Paller and Blas, 2018).

Data Collection

We installed SM4 passive acoustic recorders (PARs; Wildlife Acoustics) at nineteen of the long-term monitoring sites that are regularly sampled for aquatic macroinvertebrates and fish on the SRS (Table 1; Figure 4.1). These sites occur in three of the SRS drainage units: Upper Three Runs, Pen's Branch, and Steel Creek. Six sites are affected by non-radiological waste that includes mixtures of heavy metals, metalloids, and hazardous chemicals (USEPA, 1996). Five sites are affected by radiological waste primarily consisting of the radionuclide cesium-137 (^{137}Cs). Four sites are affected by mixed non-radiological and radiological waste, and the remaining four sites are minimally impacted by contamination.

Each PAR was installed at a height of 1.5 meters on trees with a diameter of less than 15 cm. PARs were attached to the north side of trees to limit their exposure to direct sunlight and were located at least 100 meters from active roads and out of range of stream sounds.

Acoustic sampling occurred from June 28 to August 02, 2024, which represents the midsummer period characterized by a high level of acoustic activity from birds, anurans, and insects. Each PAR recorded two hours of the dawn soundscape (sunrise ± 1 hour) and two hours of the dusk soundscape (sunset ± 1 hour) using two, built-in omnidirectional microphones. We programmed PARs to track daily sunrise and sunset times and to collect audio with a sampling rate of 44.1 kHz, a gain of 16 dB, and a preamp gain of 26 dB.

Acoustic data were segmented into one-minute samples for analysis. The use of multiple acoustic indices to investigate soundscapes is recommended as they can reveal complementary patterns (Barbaro et al., 2022). For each one-minute sample, we calculated the Bioacoustic Index (BI), Acoustic Complexity Index (ACI), Acoustic Diversity Index (ADI), and Event Count Index (EVN; Table 2). The BI, ACI, and ADI were calculated with the *soundecology* package in R (Villanueva-Rivera and Pijanowski, 2018), and the EVN was calculated in Kaleidoscope Pro (Wildlife Acoustics).

Statistical Analysis

We fit a linear mixed regression model to each of the four acoustic indices (BI, ACI, ADI and EVN) for each of the two time periods (dawn and dusk) to evaluate their utility as indicators of riparian ecosystem function. We included two fixed effects that aimed to quantify the extent of contamination within a one-kilometer radius of each PAR: hazardous waste disposal area and ^{137}Cs contamination index (Figure 4.1). First, we determined the number of hectares of hazardous waste disposal area within each 314-ha circle using data from the SRS Federal Facility Agreement (USEPA, 1996). Hazardous waste disposal areas contain radiological and/or non-radiological contaminants that were released to the environment in quantities that pose a threat to human and environmental health. Second, we obtained ^{137}Cs dose rates from an aerial radiological survey of the SRS performed in 1987 (Feimster, 1991). This survey generated contour plots of waste-derived ^{137}Cs, with isolines indicating annual dose contributions, in mrem/year, from the landscape. Annual dose rates from the 1987 survey were decay-corrected to those expected for 2022 based on the ^{137}Cs half-life of 30.17 years. The area of land, in hectares, corresponding to each dose rate was determined within a one-kilometer radius of each PAR. We calculated a ^{137}Cs contamination index for each PAR using:

$$\Sigma(d_i A_i)$$

Where *d* is the vector of decay-corrected dose rates and *A* is the area of land (ha) corresponding to each dose rate, thus producing a measure of the total dose per hectare of all ^{137}Cs-contaminated habitat within a one-kilometer radius of each PAR.

Landscape structure is known to influence both the propagation of sound and the occupancy of acoustically-active organisms (Pijanowski et al., 2011). We therefore evaluated a set of fixed effects that aimed to characterize landcover type and vegetation structure within a 200-meter radius of each PAR. Landcover data with a resolution of 30 m^2 were obtained from the 2021 National Land Cover Database (NLCD; USGS 2021). The eleven landcover categories found at our study sites were consolidated into four classes: forest (deciduous forest, evergreen forest, and mixed forest), sparse vegetation (shrub/scrub and herbaceous), wetlands (woody wetlands, emergent herbaceous wetlands, and open water) and developed land (open developed, low intensity developed, and medium intensity developed). We determined the number of hectares attributed to each landcover class within each PAR radius. We also calculated the mean canopy height and basal area of all live softwood and hardwood trees within each 200-meter radius using LiDAR products derived from a 2018 aerial survey contracted by the USDA Forest Service, Savannah River. Areas of contamination, landcover, and vegetation structure were all calculated in ArcPro (ESRI 2024). We evaluated landcover and vegetation covariates for multicollinearity by calculating Pearson's correlation coefficient for all pairwise combinations. Wetland and forest hectares were collinear ($r < -0.7$), so the latter variable was removed from the models.

Soundscapes can vary temporally over the course of a single dawn or dusk period. To account for this variation, we included a categorical fixed effect for time segment. We categorized the first, second, and third 40-minute increments of each two-hour recording as "pre-dawn/dusk", "dawn/dusk", and "post-dawn/dusk".

All statistical analyses were conducted in R. We used the *lme4* package to fit eight linear mixed regression models with the dawn and dusk values of each of the four acoustic indices (BI, ACI, ADI and EVN) as response variables and hazardous waste disposal area, ^{137}Cs contamination index, wetland area, developed land area, mean canopy height, mean basal area, and time period as fixed effects. All numerical fixed effects were standardized to have a mean of zero and a standard deviation of one. To account for repeated sampling of soundscapes across days and sites, we included random effects for minute, date, and site. We fit the full model containing all predictors to each of the eight response variables and evaluated the significance of fixed effects using an α value of 0.05. The distributions of the ADI and EVN exhibited negative and positive skew, respectively, so we fit additional LMER models to transformed versions of these indices. We transformed the ADI by reflecting over the maximum value to achieve a positive skew and applying a log-transformation, and we log-transformed the EVN. We examined the normality of the residuals for all models using the DHARMa package (Hartig, 2022) and determined that the ADI and EVN transformations improved model fit.

To compare the acoustic indices to traditional biotic indices, we fit linear mixed regression models to the two biotic indices MHSP and IBI derived from three decades of surveys at our study sites. We fit a separate model to each of the two biotic indices (MHSP and IBI) as response variables and included hazardous waste disposal area and ^{137}Cs contamination index as fixed effects. Site and year were included as random effects.

RESULTS

Nine sites were affected by ^{137}Cs contamination within a one-kilometer radius of the PAR. Decay-corrected dose rates ranged from 1.3 to 130 mrem/year. The sites with the highest ^{137}Cs contamination index occurred at lower Steel Creek (SC2 and SC3), where relatively large land areas contributed low to moderate dose rates. Despite having a small area of contamination, Craig's Branch (CB), also had a high ^{137}Cs contamination index due to the presence of high dose rates within the contamination footprint. Ten sites were affected by hazardous waste within a one-kilometer radius of the PAR. Nine of these had hazardous waste areas ranging from 2.2 to 6.2 ha (mean 3.3, SD 2.2) and the tenth, Tim's Branch (TB), had a waste area of 35 ha.

The majority of land cover within a 200m radius of all PARs was categorized as wetlands, followed by forest (Figure 4.2). Seven PARs were near small areas of land categorized as developed, all of which represented paved access roads except for at Indian Grave Branch (IGB), which was near the edge of a foundation that supported a now-demolished nuclear reactor. Few PARs were adjacent to sparsely vegetated areas except for McQueen's Branch 1 (MQ1) which was near a stand of young planted pine. Vegetation structure was consistent across sites, as indicated by the low between-site variation in the mean plot height ($\bar{x}$ 15.7 m SD 0.51 m) and mean basal area ($\bar{x}$ 3.6 m^3, SD 0.30 m^3).

All acoustic indices varied significantly over the course of each two-hour recording period but exhibited different temporal patterns (Figure 4.3; B.1). The BI, a relative measure of sound intensity in the 2-8 kHz frequency band, declined gradually over the course of the dawn period but showed a parabolic trend during the dusk period, with a peak at sunset (Figure 4.4). The ADI, a measure of sound evenness, also declined over the dawn period but increased over the dusk period. Both the ACI, which measures temporal complexity, and the EVN, which counts discrete acoustic events, increased over the dawn period and declined over the dusk period (Table B.1; B.2).

Hazardous waste disposal area was a significant predictor of the BI, but the direction of the effect differed between the dawn and dusk time periods (Figure 5). Sites affected by greater areas of hazardous waste had significantly lower dawn BI values (β -0.42, SE 0.15, $p = 0.01$) but higher dusk BI values (β 0.54, SE 0.21, $p = 0.02$) compared to unaffected sites. Visual inspection of the BI models revealed that data from TB had high leverage due to the large area of hazardous waste. We examined the influence of TB on the effects of hazardous waste area by excluding it and refitting the BI models. The exclusion of TB did not alter the strength of the evidence for the positive effect of hazardous waste on dusk BI (β 1.78, SE 0.71, $p = 0.02$) but increased the uncertainty for the negative effect on dawn BI (β -0.95, SE 0.55, $p = 0.10$; Figure 5).

None of the acoustic indices were significantly predicted by the ^{137}Cs contamination index or by developed land area. Wetland area had a significant, positive correlation with dusk ACI (β 1.77, SE 0.78, p = 0.03) and a near-significant correlation with dawn ACI (β 2.32, SE 1.13, p = 0.05). Mean plot height was a predictor of the dusk ADI (β 0.50, SE 0.17, p = 0.008). Mean basal area was not a significant predictors of any acoustic indices.

The acoustic indices varied considerably among individual sites and dates, as indicated by the high random effects variance (Table B.1; B.2). Based on the conditional R^2 values for the dawn and dusk periods, the full models accounted for 26% and 20% of the variation in EVN, 34% and 40% of the variation in BI, 44% and 26% of the ADI, and 48% and 49% of the ACI.

The multihabitat sampling protocol (MHSP) score, based on historical aquatic macroinvertebrate assemblages, had a significant, negative correlation with hazardous waste disposal area (β -0.47, SE 0.11, p < 0.001; Table B.3). The effect of hazardous waste on MHSP remained marginally significant after the exclusion of data from TB (β -0.83, SE 0.38, p = 0.05). There was no relationship between MHSP score and ^{137}Cs contamination index. The Index of Biotic Integrity (IBI), based on historical fish assemblages, was not predicted by hazardous waste or ^{137}Cs index.

DISCUSSION

This study investigated the use of four acoustic indices—BI, ACI, ADI, and EVN—to detect riparian soundscape changes associated with chemical disturbance from coal combustion and nuclear fission legacy wastes. The BI alone was related to the quantity of hazardous waste within a one-kilometer radius of streamside monitoring locations on the SRS. Our findings suggest that environmental contamination from legacy waste disposal disrupts riparian ecosystem function in a manner that alters the production of acoustic energy and is detectable by the BI, but not by the ACI, ADI, and EVN.

Among the four acoustic indices considered here, the BI is the only one that quantifies the intensity of acoustic energy across frequency ranges (Boelman et al., 2007, Bradfer-Lawrence et al., 2024). For a single recording, the BI is derived by calculating the mean amplitudes of sound in each frequency bin, subtracting the lowest mean amplitude from each value, and summing the values (Bradfer-Lawrence et al., 2024). The BI was originally developed to target birdsong and is thus calculated across the 2 to 8 kHz range, which excludes low-frequency anthropogenic noise and some high-frequency insect sound (Boelman et al., 2007). Low BI values occur when fewer frequency bins within the relevant range are occupied by sound or when amplitudes are even across bins. Alternatively, high BI values occur when most of the acoustic space is occupied and when there are high amplitude disparities across bins. The lower dawn BI at hazardous waste sites suggests that their dawn chorus soundscapes are characterized by lower-intensity biological sounds in one or more frequency bands within the 2 to 8 kHz range, whereas the higher dusk BI indicates the opposite phenomenon for dusk chorus soundscapes. Despite changes in acoustic intensity, the absence of correlations between hazardous waste and the other indices suggests that the evenness of acoustic energy across frequency bins, temporal complexity, and number of sounds do not differ significantly at sites affected by hazardous waste.

The opposing directions of the changes in the dawn and dusk BI at sites affected by hazardous wastes indicate that the distinct acoustic communities comprising these two periods have different responses to environmental contamination. Dawn is the primary window for bird chorus activity, and a reduction in the dawn BI may occur due to changes in the abundance or richness of the songbird community. Although the habitat structure of waste sites was comparable to that of minimally impacted sites in this study, their altered environmental chemistry may disrupt critical flows of energy and biomass and limit their ability to support abundant avian communities (Schulz et al., 2015). In a landscape-scale evaluation of contaminant impacts on wildlife populations, Carlsen et al. (2004) suggested that localized contamination can be modeled as a form of habitat fragmentation, where contaminated areas become unusable and wildlife are relegated to surrounding patches. If birds preferentially remain in adjacent, higher-quality habitat, a lower abundance of individuals in the immediate vicinity of the PAR would lead to fewer instances of high-amplitude sound, either due to weaker chorusing activity or a lower likelihood of individual sound production at close proximity to PAR microphones. Prior studies suggest that the BI is more sensitive to changes in avian species richness than in anuran and insect richness due to the frequency range over which it is calculated (Bradfer-Lawrence et al., 2020, Eldridge et al., 2018, Ross et al., 2021, Sueur et al., 2008). Therefore, the loss of a few key bird species from hazardous waste sites could lead to a loss in sound intensity that drives down the BI. Alternatively, individuals at hazardous waste sites may spend less time producing sound than those at minimally impacted sites due to the adverse effects of contaminant uptake on their health (Casagrande et al., 2016). Songbirds are known to accumulate potentially toxic trace elements and radionuclides from hazardous waste areas on the SRS, with riparian habitat specialists having the highest rates of uptake (Werner et al., 2025). Participation in the dawn chorus is energetically expensive and an honest indicator of songbird health, and impaired song performance due to contaminant toxicity has been observed in both field and laboratory conditions (Goodchild et al., 2021, Gorissen et al., 2005). Notably, all of these mechanisms could occur simultaneously at hazardous waste sites, contributing to an overall, impaired state of ecological function that is detectable via a reduction in the intensity of the dawn biological soundscape.

In contrast to the dawn soundscapes, the dusk soundscapes of sites affected by hazardous waste had higher values of the BI, indicating higher-amplitude biological sound. Although bird chorus activity contributes to the dusk soundscape, dusk coincides with heightened acoustic activity among nocturnal insects in tropical and subtropical zones (Scarpelli et al., 2023, Stanley et al., 2016). Several orthopteran species native to the southeastern United States, including the common true katydid (*Pterophylla camellifolia*), jumping bush cricket (*Orocharis saltator*), and Say's trig (*Anaxipha exigua*), produce songs within the 2 to 8 kHz range that can dominate dusk recordings (Franklin et al., 2009, Walker, 1969, Walker and Funk, 2014). In general, loudness of orthopteran chorusing is associated with population size (Fischer et al., 1997, Penone et al., 2013). Higher dusk BI values at hazardous waste sites may therefore indicate an increase in the abundance of one or more orthopteran species, though this is counterintuitive given that prior studies conducted on the SRS have demonstrated heavy metal accumulation among terrestrial arthropods, including ground-dwelling crickets (Gryllidae; Holland et al. 2025, O'Quinn et al. 2005, Silva et al. 2023). However, community-scale studies of arthropod assemblages indicate that varying sensitivities to habitat degradation often favor a small number of species with lower susceptibility or higher tolerance (Chisté et al., 2016, Migliorini et al., 2004). Disparate responses to environmental stressors alter competitive and predatory dynamics which can further contribute to species dominance in disturbed settings (Gardiner and Harwood, 2017). The differential sensitivities of aquatic macroinvertebrates to chemical pollutants are relatively well-known, and thus a lower richness of ephemeropterans, plecopterans, and tricopterans, corresponding to a dominance of dipterans, is considered a reliable indicator of polluted habitat (Winner et al., 1980). In comparison, the relative sensitivity of orthopterans and other terrestrial arthropods to environmental pollution is poorly understood. Katydids (Tettigoniidae) and trigs (Trigonidiidae) are primarily arboreal and may have lower exposure to legacy contaminants that persist in soil and sediment compared to ground-dwelling and aquatic invertebrates (Fletcher et al., 2019, Sullivan and Rodewald, 2012). Additionally, as herbivores, they accumulate lower concentrations of biomagnifying contaminants than the arachnids, birds, and small fossorial mammals that prey upon them (Hulbert et al., 2021, Mogren and Trumble, 2010, Zhang et al., 2022). We speculate that the differential sensitivities of riparian taxa to chemical disturbance drive changes in community assemblages that are detectable via opposing shifts in the intensity of the dawn and dusk choruses. Specifically, key nocturnal orthopterans may exhibit low sensitivity to contamination in riparian habitat and may benefit from a decrease in competition or predation stemming from the loss of more sensitive taxa, including songbird predators. Ultimately, follow-up studies that quantify species occupancy and abundance are required to identify how specific shifts in community assemblages lead to ecosystem-scale changes in collective sound production at contaminated sites.

We hypothesized that the degradation of riparian soundscapes would coincide with that of aquatic biodiversity, and thus we expected that patterns in acoustic indices would reflect those of traditional biotic indices across contaminated sites. We found that both the MHSP and the dawn BI, though not the dusk BI, were inversely related to hazardous waste area. Therefore, streams affected by larger areas of hazardous waste have a lower diversity of sensitive, aquatic macroinvertebrates, and their surrounding riparian habitats are characterized by a lower-intensity dawn chorus. Freshwater environments are hotspots of resource exchange that support ecosystem function at landscape scales (Schindler and Smits, 2016). The degree to which contaminants interfere with these functions depends on their mobility along resource exchange pathways, bioavailability to organisms, and toxicity potential (Sullivan and Rodewald, 2012). In the present study, the absence of select biota from both aquatic habitat and riparian soundscapes suggests that community-scale impacts of legacy contamination persist decades after waste input to freshwater systems. This finding is supported by studies that have demonstrated ongoing contaminant transfer from aquatic to terrestrial systems at concentrations that surpass thresholds of concern for reproductive effects in riparian taxa (Edwards et al., 2014, Fletcher, 2022, Werner et al., 2025). By altering the diversity and abundance of aquatic biota, contaminants also disrupt prey availability for riparian predators and indirectly modulate the structure and function of terrestrial communities (Schulz et al., 2015). Reductions in aquatic invertebrate biomass are associated with lower abundances of several riparian taxa, including ground beetles (Paetzold et al., 2005), spiders (Kato et al., 2003), birds (Mattsson and Cooper, 2006), and bats (Fukui et al., 2006). Our findings demonstrate the utility of combining passive acoustic monitoring with traditional biodiversity surveys to assess linkages in the disturbance responses of aquatic and terrestrial communities and conduct multimetric assessments of contaminant impacts to their shared ecosystems.

The observed trends in BI and MHSP at hazardous waste sites were largely driven by the values of both indices at Tim's Branch, which has the largest area of waste among all sites. Between 1954 and 1985, approximately 43,500 kg of uranium-238 (U) were discharged into Tim's Branch, resulting in sediment concentrations that are 3,000 times higher than background concentrations in the southeastern USA (Pickett, 1990). Other elevated contaminants at Tim's Branch include nickel, chromium, copper, thorium, lead and mercury (Edwards et al., 2014). Long-term surveys of biota at Tim's Branch indicate that metals are bioavailable and transferred to both aquatic and riparian organisms, suggesting widespread impairment of ecological integrity (Edwards et al., 2014, Paller and Blas, 2018, Punshon et al., 2009).

Therefore, the low, average dawn BI values observed in this study are consistent with prior observations that identify Tim's Branch as having the greatest long-term impact due to metals contamination. Alternatively, we did not observe a difference in acoustic indices at sites primarily affected by the radionuclide ^{137}Cs. Although bioaccumulation of ^{137}Cs has been observed on the SRS, transfer rates to biota are relatively low due to its strong adsorption to sediments and limited bioavailability (Kennamer et al., 2017, Leaphart et al., 2020, Oldenkamp et al., 2017, Werner et al., 2025). Our findings are consistent with those of Paller et al. (2018), who concluded that the ecological integrity at sites affected by ^{137}Cs has generally recovered following the cessation of thermal pollution from nuclear reactor effluent.

The riparian sites in this study had minimal structural disturbance and consisted primarily of woody wetlands and pine forests. Forest structure affects a complex array of factors that influence soundscapes, including the composition of acoustically active communities, the propagation of sound, and physical conditions like temperature and light that stimulate acoustic activity (Barbaro et al. 2022). In this study, mean basal area and canopy height were not consistent predictors of acoustic index values, likely due to the low between-site variation in forest structure. The soundscapes of sites with a higher proportion of wetland landcover had higher ACI values, indicating greater temporal complexity. The ACI increases when acoustic space is occupied by the variable-amplitude songs of birds, rhythmic anurans, and rhythmic insects but remains insensitive to constant-amplitude sound produced by anthropogenic noise and some organisms (e.g. trilling insects; Ross et al., 2021; Turlington et al., 2024) . Our findings indicate that the ACI is a sensitive index for detecting the unique acoustic signature of wetland ecosystems. Overall, the use of multiple acoustic indices and the incorporation of vegetation covariates in this study allowed us to distinguish between the differences in sound complexity associated with habitat variation and the changes in sound intensity associated with the presence of hazardous waste.

CONCLUSION

The biological production of acoustic energy on a landscape serves an ecosystem-scale endpoint that can indicate responses to environmental change. This study evaluated the use of acoustic indices to identify differences in ecosystem function across nineteen riparian sites affected by varying quantities of both radiological and non-radiological hazardous legacy wastes. We found that the intensity of biological sound, as measured by the BI, was altered in habitat affected by hazardous waste. The observed decrease in dawn BI values at hazardous waste sites reflected those of biotic integrity scores derived from aquatic macroinvertebrate surveys, suggesting that reduced dawn chorus activity in the riparian zone is linked to the degradation of aquatic habitat. Our findings demonstrate that midsummer dawn and dusk soundscapes are sensitive indicators of altered, riparian ecosystem function in a contaminated, subtropical environment. In the future, passive acoustic monitoring may be a cost effective and efficient means of monitoring longer-term trends in ecosystem response to waste influx, seasonal or annual patterns in contaminant cycling, and outcomes of remediation initiatives.

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LITERATURE CITED

Barbaro, L., Sourdril, A., Froidevaux, J. S. P., Cauchoix, M., Calatayud, F., Deconchat, M. & Gasc, A. 2022. Linking acoustic diversity to compositional and configurational heterogeneity in mosaic landscapes. *Landscape Ecology,* 37**,** 1125-1143.

Bargagli, R. 2008. Environmental contamination in Antarctic ecosystems. *Science of The Total Environment,* 400**,** 212-226. https://doi.org/10.1016/j.scitotenv.2008.06.062

Becker, D. J., Chumchal, M. M., Broders, H. G., Korstian, J. M., Clare, E. L., Rainwater, T. R., Platt, S. G., Simmons, N. B. & Fenton, M. B. 2018. Mercury bioaccumulation in bats reflects dietary

connectivity to aquatic food webs. *Environmental Pollution,* 233**,** 1076-1085. https://doi.org/10.1016/j.envpol.2017.10.010
Boelman, N. T., Asner, G. P., Hart, P. J. & Martin, R. E. 2007. Multi-trophic invasion resistance in Hawaii: bioacoustics, field surveys, and airborne remote sensing. *Ecological Applications,* 17**,** 2137-2144. https://doi.org/10.1890/07-0004.1
Bradfer-Lawrence, T., Bunnefeld, N., Gardner, N., Willis, S. G. & Dent, D. H. 2020. Rapid assessment of avian species richness and abundance using acoustic indices. *Ecological Indicators*. https://doi.org/10.1016/j.ecolind.2020.106400
Bradfer-Lawrence, T., Duthie, B., Abrahams, C., Adam, M., Barnett, R. J., Beeston, A., Darby, J., Dell, B., Gardner, N., Gasc, A., Heath, B., Howells, N., Janson, M., Kyoseva, M.-V., Luypaert, T., Metcalf, O. C., Nousek-McGregor, A. E., Poznansky, F., Ross, S. R. P.-J., Sethi, S., Smyth, S., Waddell, E. & Froidevaux, J. S. P. 2024. The acoustic index user's guide: a practical manual for defining, generating, and understanding current and future acoustic indices. *Methods in Ecology and Evolution*. doi.org/10.1111/2041-210X.14357
Burivalova, Z., Purnomo, Wahyudi, B., Boucher, T. M., Ellis, P., Truskinger, A., Towsey, M., Roe, P., Marthinus, D., Griscom, B. & Game, E. T. 2019. Using soundscapes to investigate homogenization of tropical forest diversity in selectively logged forests. *Journal of Applied Ecology,* 56**,** 2493-2504. https://doi.org/10.1111/1365-2664.13481
Carlsen, T. M., Coty, J. D. & Kercher, J. R. 2004. The spatial extent of contamination and the landscape scale: an analysis of the wildlife, conservation biology, and population modeling literature. *Environmental Toxicology and Chemistry,* 23**,** 798-811. https://doi.org/10.1897/02-202
Carlton, W. H., Bauer, L. R., Evans, A. G., Geary, L. A., Murphy Jr., C. E., Pinder, J. E. & Strom, R. N. 1992. Cesium in the Savannah River Site environment. U.S. Department of Energy.
Casagrande, S., Pinxten, R. & Eens, M. 2016. Honest Signaling and Oxidative Stress: The Special Case of Avian Acoustic Communication. *Frontiers in Ecology and Evolution,* 4. https://doi.org/10.3389/fevo.2016.00052
Chisté, M. N., Mody, K., Gossner, M. M., Simons, N. K., Köhler, G., Weisser, W. W. & Blüthgen, N. 2016. Losers, winners, and opportunists: How grassland land-use intensity affects orthopteran communities. *Ecosphere,* 7**,** e01545. https://doi.org/10.1002/ecs2.1545
Clark, A. J., Labaj, A. L., Smol, J. P., Campbell, L. M. & Kurek, J. 2021. Arsenic and mercury contamination and complex aquatic bioindicator responses to historical gold mining and modern watershed stressors in urban Nova Scotia, Canada. *Science of The Total Environment,* 787**,** 147374. https://doi.org/10.1016/j.scitotenv.2021.147374
Duan, R.-Y. & Huang, M.-Y. 2016. The influence of low-dose cadmium on the laryngeal microstructure and ultrastructure of *Pelophylax nigromaculata*. *Environmental Science and Pollution Research,* 23**,** 17322-17331.
Dwivedi, D., Steefel, C. I., Arora, B., Banfield, J., Bargar, J., Boyanov, M. I., Brooks, S. C., Chen, X., Hubbard, S. S., Kaplan, D., Kemner, K. M., Nico, P. S., O'Loughlin, E. J., Pierce, E. M., Painter, S. L., Scheibe, T. D., Wainwright, H. M., Williams, K. H. & Zavarin, M. 2022. From legacy contamination to watershed systems science: a review of scientific insights and technologies developed through DOE-supported research in water and energy security. *Environmental Research,* 17. https://doi.org/10.1088/1748-9326/ac59a9
Edwards, P. G., Gaines, K. F., Bryan Jr., A. L., Novak, J. M. & Blas, S. A. 2014. Trophic dynamics of U, Ni, Hg and other contaminants of potential concern on the Department of Energy's Savannah River Site. *Environmental Monitoring and Assessment,* 186**,** 481-500. https://doi.org/10.1007/s10661-013-3392-z
Eldridge, A., Guyot, P., Moscoso, P., Johnston, A., Eyre-Walker, Y. & peck, M. 2018. Sounding out ecoacoustic metrics: Avian species richness is predicted by acoustic indices in temperate but not tropical habitats. *Ecological Indicators,* 95**,** 939-952. https://doi.org/10.1016/j.ecolind.2018.06.012

Feimster, E. L. 1991. An aerial radiological survey of the Central Savannah River Site, Aiken, South Carolina. https://doi.org/10.2172/5031957
Ferrari, C. R., do Nascimento, H. d. A. F., Rodgher, S., Almeida, T., Bruschi, A. L., do Nascimento, M. R. L. & Bonifácio, R. L. 2017. Effects of the discharge of uranium mining effluents on the water quality of the reservoir: an integrative chemical and ecotoxicological assessment. *Scientific Reports,* 7**,** 13919. https://doi.org/10.1038/s41598-017-14100-w
Fischer, F. P., Schulz, U., Schubert, H., Knapp, P. & Schmöger, M. 1997. Quantitative assessment of grassland quality: acoustic determination of population sizes of orthopteran indicator species. *Ecological Applications,* 7**,** 909-920. https://doi.org/10.1890/1051-0761(1997)007[0909:QAOGQA]2.0.CO;2
Fletcher, D. E. 2022. Species- and element-specific patterns of metal flux from contaminated wetlands versus metals shed with exuviae in emerging dragonflies. *Environmental Pollution,* 300. https://doi.org/10.1016/j.envpol.2022.118976
Fletcher, D. E., Lindell, A. H., Seaman, J. C., Stankus, P. T., Fletcher, N. D., Barton, C. D., Biemiller, R. A. & McArthur, J. V. 2019. Sediment and biota trace element distribution in streams disturbed by upland industrial activity. *Environmental Toxicology and Chemistry,* 38**,** 115-131. https://doi.org/10.1002/etc.4287
Fletcher, D. E., Lindell, A. H., Stillings, G. K., Mills, G. L., Blas, S. A. & McAurthur, J. V. 2014. Spatial and taxonomic variation in trace element bioaccumulation in two herbivores from a coal combustion waste contaminated stream. *Ecotoxicology and Environmental Safety,* 101.
Franklin, M., Droege, S., Dawson, D. & Royle, J. A. 2009. Nightly and seasonal patterns of calling in common true katydids (Orthoptera: Tettigoniidae: *Pterophylla camellifolia*). *Journal of Orthoptera Research,* 18**,** 15-18. https://doi.org/10.1665/034.018.0108
Fukui, D., Murakami, M., Nakano, S. & Aoi, T. 2006. Effect of emergent aquatic insects on bat foraging in a riparian forest. *Journal of Animal Ecology,* 75**,** 1252-1258. https://doi.org/10.1111/j.1365-2656.2006.01146.x
Fulghum, C. M., DiBona, E. R., Leaphart, J. C., Korotasz, A. M., Beasley, J. C. & Bryan Jr., A. L. 2019. Radiocesium (^{137}Cs) accumulation by fish within a legacy reactor cooling canal system on the Savannah River Site. *Environment International,* 126**,** 216-221. https://doi.org/10.1016/j.envint.2019.02.039
Gardiner, M. M. & Harwood, J. D. 2017. Influence of heavy metal contamination on urban natural enemies and biological control. *Current Opinion in Insect Science,* 20**,** 45-53. http://dx.doi.org/10.1016/j.cois.2017.03.007
Gasc, A., Sueur, J., Jiguet, F., Devictor, V., Grandcolas, P., Burrow, C., Depraetere, M. & Pavoine, S. 2013. Assessing biodiversity with sound: Do acoustic diversity indices reflect phylogenetic and functional diversities of bird communities? *Ecological Indicators,* 25**,** 279-287.
Gewurtz, S. B., Backus, S. M., Bhavsar, S. P., McGoldrick, D. J., de Solla, S. R. & Murphy, E. W. 2011. Contaminant biomonitoring programs in the Great Lakes region: Reviews of approaches and critical factors. *Environmental Reviews,* 19**,** 162-184.
Goodchild, C. G., Beck, M. L., VanDiest, I., Czesak, F. N., Lane, S. J. & Sewall, K. B. 2021. Male zebra finches exposed to lead (Pb) during development have reduced volume of song nuclei, altered sexual traits, and received less attention from females as adults. *Ecotoxicology and Environmental Safety,* 210**,** 111850. https://doi.org/10.1016/j.ecoenv.2020.111850
Gorissen, L., Snoeijs, T., Duyse, E. V. & Eens, M. 2005. Heavy metal pollution affects dawn singing behaviour in a small passerine bird. *Oecologia,* 145**,** 504-509. 10.1007/s00442-005-0091-7
Grinfeder, E., Haupert, S., Ducrettet, M., Barlet, J., Reynet, M., Sébe, F. & Sueur, J. 2022. Soundscape dynamics of a cold protected forest: dominance of aircraft noise. *Landscape Ecology,* 37**,** 567-582. https://doi.org/10.1007/s10980-021-01360-1
Hartig, F. 2022. DHARMa: Residual diagnostics for hierarchical (multi-level/mixed) regression models.

Holland, K. E., Tuberville, T. D. & Beasley, J. C. 2025. Accumulation of trace elements in soil and fauna within a site historically contaminated with coal combustion residues. *Environmental Toxicology and Chemistry,* 44**,** 1039-1050. https://doi.org/10.1093/etojnl/vgaf020

Hulbert, A. H., Olsen, A. M., Sawyer, M. M. & Winner, P. M. 2021. The Avian Diet Database as a source of quantitative information on avian deits. *Scientific Data,* 8. https://doi.org/10.1038/s41597-021-01049-9

Kato, C., Iwata, T., Nakano, S. & Kishi, D. 2003. Dynamics of aquatic insect flux affects distribution of riparian web-building spiders. *Oikos,* 103**,** 113-120. https://doi.org/10.1034/j.1600-0706.2003.12477.x

Kennamer, R. A., Oldenkamp, R. E., Leaphart, J. C., King, J. D., Bryan Jr., A. L. & Beasley, J. C. 2017. Radiocesium in migratory aquatic game birds using contaminated U.S. Department of Energy reactor-cooling reservoirs: A long-term perspective. *Journal of Environmental Radioactivity,* 171**,** 189-199. http://dx.doi.org/10.1016/j.jenvrad.2017.02.022

Leaphart, J. C., Korotasz, A. M., Bryan Jr., A. L. & Beasley, J. C. 2020. Environmental fate of radiocesium in biota inhabiting a contaminated ecosystem on the U.S. Department of Energy's Savannah River Site. *Journal of Environmental Radioactivity,* 222. https://doi.org/10.1016/j.jenvrad.2020.106321

Leaphart, J. C., Wilms, K. C., Bryan Jr., A. L. & Beasley, J. C. 2019. Bioaccumulation of 137Cs in anuran larvae utilizing a contaminated effluent canal on the U.S. Department of Energy's Savannah River Site. *Journal of Environmental Radioactivity,* 203**,** 25-29. https://doi.org/10.1016/j.jenvrad.2019.02.012

Mason, C. F. 2002. *Biology of freshwater pollution,* Harrow, England, Prentice Hall.

Mattsson, B. J. & Cooper, R. J. 2006. Louisiana waterthrushes (*Seiurus motacilla*) and habitat assessments as cost-effective indicators of instream biotic integrity. *Freshwater Biology,* 51**,** 1941-1958. https://www.doi.org/10.1111/j.1365-2427.2006.01617.x

Migliorini, M., Pigino, G., Bianchi, N., Bernini, F. & Leonzio, C. 2004. The effects of heavy metal contamination on the soil arthropod community of a shooting range. *Environmental Pollution,* 129**,** 331-340. https://doi.org/10.1016/j.envpol.2003.09.025

Mogren, C. L. & Trumble, J. T. 2010. The impacts of metals and metalloids on insect behavior. *Entomologia Experimentalis et Applicata,* 135**,** 1-17. https://www.doi.org/10.1111/j.1570-7458.2010.00967.x

Moller, A. P. & Mousseau, T. A. 2007. Species richness and abundance of forest birds in relation to radiation at Chernobyl. *Biology Letters*.

O'Quinn, G. N. 2005. *Using terrestrial arthropods as receptor species to determine trophic transfer of heavy metals in a riparian ecosystem.* MS Thesis, University of Georgia.

Oldenkamp, R. E., Bryan Jr., A. L., Kennamer, R. A., Leaphart, J. C., Webster, S. C. & Beasley, J. C. 2017. Trace elements and radiocesium in game species near contaminated sites. *The Journal of Wildlife Management,* 81**,** 1338-1350. https://doi.org/10.1002/jwmg.21314

Paetzold, A., Schubert, C. J. & Tockner, K. 2005. Aquatic terrestrial linkages along a braided-river. *Ecosystems,* 8**,** 748-759. https://doi.org/10.1007/s10021-005-0004-y

Paller, M. H. & Blas, S. A. 2018. Bioassessment of streams on the Savannah River Site, 1990 to 2017.

Paller, M. H., Martin, F. D., Wike, L. D. & Specht, W. L. 2007. Factors influencing the accuracy of a macroinvertebrate bioassessment protocol in South Carolina coastal plain streams. *Journal of Freshwater Ecology,* 22**,** 23-32. https://doi.org/10.1080/02705060.2007.9664142

Paller, M. H., Reichert, M. J. M. & Dean, J. M. 1996. Use of fish communities to assess environmental impacts in South Carolina coastal plain streams. *Transactions of the American Fisheries Society*. https://doi.org/10.1577/1548-8659(1996)125<0633:UOFCTA>2.3.CO;2

Penone, C., Le Viol, I., Pellissier, V., Jean-Francois, J., Bas, Y. & Kerbiriou, C. 2013. Use of large-scale acoustic monitoring to assess anthropogenic pressures on Orthoptera communities. *Conservation Biology,* 27**,** 979-987. https://www.jstor.org/stable/23525549

Phillips, D. J. H. & Rainbow, P. S. 1994. *Biomonitoring of Trace Aquatic Contaminants*, Springer Netherlands.
Pickett, J. B. 1990. Heavy metal contamination in Tims Branch sediments. Westinghouse Savannah River Company.
Pijanowski, B. C. 2024. *Principles of Soundscape Ecology,* Chicago, USA, The University of Chicago Press.
Pijanowski, B. C., Villanueva-Rivera, L. J., Dumyahn, S. L., Farina, A., Krause, B. L., Napoletano, B. M., Gage, S. H. & Pieretti, N. 2011. Soundscape Ecology: The Science of Sound in the Landscape. *BioScience,* 61**,** 203-216.
Punshon, T., Gaines, K. F., Bertsch, P. M. & Burger, J. 2009. Bioavailability of uranium and nickel to vegetation in a contaminated riparian ecosystem. *Environmental Toxicology and Chemistry,* 22**,** 1146-1154. https://doi.org/10.1002/etc.5620220525
Rhodes, O. E., Bréchignac, F., Bradshaw, C., Hinton, T. G., Mothersill, C., Arnone III, J. A., Aubrey, D. P., Barnthouse, L. W., Beasley, J. C., Bonisoli-Alquati, A., Boring, L. R., Bryan, A. L., Capps, K. A., Clément, B., Coleman, A., Condon, C., Coutelot, F., DeVol, T., Dharmarajan, G., Fletcher, D. E., Flynn, W., Gladfelder, G., Glenn, T. C., Hendricks, S., Ishida, K., Jannik, T., Kapustka, L., Kautsky, U., Kennamer, R. A., Kuhne, W., Lance, S. L., Laptyev, G., Love, C., Manglass, L., Martinez, N., Mathews, T., McKee, A., McShea, W., Mihok, S., Mills, G., Parrott, B., Powell, B., Pryakhin, E., Rypstra, A., Scott, D., Seaman, J., Seymour, C., Shkvyrya, M., Ward, A., White, D., Wood, M. D. & Zimmerman, J. K. 2020. Integration of ecosystem science into radioecology: A consensus perspective. *Science of The Total Environment,* 740**,** 140031. https://doi.org/10.1016/j.scitotenv.2020.140031
Rosenberg, D. M. & Resh, V. H. 1993. *Freshwater Biomonitoring and Benthic Macroinvertebrates,* New York, New York, USA, Chapman & Hall.
Ross, S. R. P.-J., Friedman, N. R., Yoshimura, M., Yoshida, T., Donohue, I. & Economo, E. P. 2021. Utility of acoustic indices for ecological monitoring in complex sonic environments. *Ecological Indicators,* 121**,** 107114. https://doi.org/10.1016/j.ecolind.2020.107114
Rozell, D. J. & Reaven, S. J. 2011. Water pollution risk associated with natural gas extraction from the Marcellus Shale. *Risk Analysis,* 32**,** 1382-1393. https://doi.org/10.1111/j.1539-6924.2011.01757.x
Scarpelli, M. D. A., Roe, P., Tucker, D. & Fuller, S. 2023. Soundscape phenology: The effect of environmental and climactic factors on birds and insects in a subtropical woodland. *Science of The Total Environment,* 878**,** 163080. http://dx.doi.org/10.1016/j.scitotenv.2023.163080
SCDES. 1998. Standard operating and quality control procedures for macroinvertebrate sampling.
Schindler, D. E. & Smits, A. P. 2016. Subsidies of aquatic resources in terrestrial ecosystems. *Ecosystems,* 20**,** 78-93. https://doi.org/10.1007/s10021-016-0050-7
Schulz, R., Bundschuh, M., Gergs, R., Brühl, C. A., D., D., Entling, M. H., Fahse, L., Frör, O., Jungkunst, H. F., Lorke, A., Schäfer, R. B., Schaumann, G. E. & Schwenk, K. 2015. Review on environmental alterations propagating from aquatic to terrestrial ecosystems. *Science of The Total Environment,* 538**,** 246-261. http://dx.doi.org/10.1016/j.scitotenv.2015.08.038
Sievers, M., Hale, R., Parris, K. M. & Swearer, S. E. 2018. Impacts of human-induced environmental change in wetlands on aquatic animals. *Biological Reviews,* 93**,** 529-554. https://doi.org/10.1111/brv.12358
Silva, A. E., Speakman, R. J., Barns, B. F., Coyle, D. R., Leaphart, J. C., Abernethy, E. F., Turner, K. L., Rhodes Jr., O. E., Beasley, J. C. & Gandhi, K. J. K. 2023. Bioaccumulation of contaminants in Scarabidae and Silphidae beetles at sites polluted by coal combustion residuals and radiocesium. *Science of The Total Environment,* 904**,** 166821. https://doi.org/10.1016/j.scitotenv.2023.166821
Smalling, K. L., Anderson, C. W., Honeycutt, R. K., Cozzarelli, I. M., Preston, T. & Hossack, B. R. 2019. Associations between environmental pollutants and larval amphibians in wetlands contaminated by energy-related brines are potentially mediated by feeding traits. *Environmental Pollution,* 248**,** 260-268.

SRNS. 2020. Comprehensive alternative evaluation of ash and coal fine waste units at the Savannah River Site.
Stanley, C. Q., Walter, M. H., Venkatraman, M. X. & Wilkinson, G. S. 2016. Insect noise avoidance in the dawn chorus of Neotropical birds. *Animal Behavior,* 112**,** 255-265. http://dx.doi.org/10.1016/j.anbehav.2015.12.003
Stapanian, M. A., Micacchion, M. & Adams, J. V. 2015. Wetland habitat disturbance best predicts metrics of an amphibian index of biotic integrity. *Ecological Indicators,* 56**,** 237-242. https://doi.org/10.1016/j.ecolind.2015.04.005
Sueur, J., Pavoine, S., Hamerlynck, O. & Duvail, S. 2008. Rapid acoustic survey for biodiversity appraisal. *PLoS ONE,* 3.
Sullivan, S. M. P. & Rodewald, A. D. 2012. The energetic pathways that move contaminants from aquatic to terrestrial environments. *Environmental Toxicology and Chemistry,* 31**,** 1175-1183.
Turlington, K., Suárez-Castro, A. F., Teixeira, D., Linke, S. & Sheldon, F. 2024. Exploring the relationship between the soundscape and the environment: A systematic review. *Ecological Indicators,* 166. https://doi.org/10.1016/j.ecolind.2024.112388
USEPA. 1996. Federal Facility Agreement for the Savannah River Site. https://doi.org/10.1080/02705060.2007.9664142
Villanueva-Rivera, L. J. & Pijanowski, B. C. 2018. Soundecology: soundscape ecology. R package ed.
Walker, T. J. 1969. Systematics and acoustic behavior of United States crickets of the genus *Orocharis* (Orthoptera: Gryllidae). *Annals of the Entomological Society of America,* 62**,** 752-762.
Walker, T. J. & Funk, D. H. 2014. Systematics and acoustics of North American *Anaxipha* (Gryllidae: Trigonidiinae). *Journal of Orthoptera Research,* 23**,** 1-38. https://doi.org/10.1665/034.023.0102
Walters, C. R., Somerset, V. S., Leaner, J. J. & Nel, J. M. 2011. A review of mercury pollution in South Africa: Current status. *Journal of Environmental Science and Health,* 46**,** 1129-1137. https://doi.org/10.1080/10934529.2011.590729
Werner, C. S., Chapman, M., Rhodes Jr., O. E. & DeVault, T. L. 2025. Uptake of metals, metalloids, and radiocesium varies with habitat use among passerine communities at coal combustion and nuclear fission legacy waste sites. *Environmental Pollution,* 367**,** 125608. https://doi.org/10.1016/j.envpol.2024.125608
White, D. L. & Gaines, K. F. 2000. The Savannah River Site: Site description, land use and management history. *Avian Research at the Savannah River Site: A Model for Integrating Basic Research and Long-Term Management.* Scholarly Commons.
Winner, R. W., Boesel, M. W. & Farrell, M. P. 1980. Insect community structure as an index of heavy-metal pollution in lotic ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences,* 37**,** 647-655. https://doi.org/10.1139/f80-081
Yang, J., Li, J., van Vliet, M. T. H., Jones, E. R., Huang, Z., Liu, M. & Bi, J. 2024. Economic risks hidden in local water pollution and global markets: A retrospective analysis (1995-2010) and future perspectives on sustainable development goal 6. *Water Research,* 252**,** 121216. https://doi.org/10.1016/j.watres.2024.121216
Zhang, F., Xu, Z., Xu, X., Liang, L., Chen, Z., Dong, X., Luo, K., Dinis, F. & Qiu, G. 2022. Terrestrial mercury and methylmercury bioaccumulation and trophic transfer in subtropical urban forest food webs. *Chemosphere,* 299. https://doi.org/10.1016/j.chemosphere.2022.134424
Zhou, M., Li, X., Zhang, M., Liu, B., Zhang, Y., Gao, Y., Ullah, H., Peng, L., He, A. & Yu, H. 2020. Water quality in a worldwide coal mining city: A scenario in water chemistry and health risks exploration. *Journal of Geochemical Exploration,* 213**,** 106513. https://doi.org/10.1016/j.gexplo.2020.106513

Table 4.1. Long-term monitoring sites across three SRS drainage units, and their contaminant histories. The primary element present at radiological waste sites is cesium-137, whereas non-radiological waste sites contain mixtures of heavy metals and metalloids. Mixed waste sites contain both radiological and non-radiological wastes. Sites were labelled as minimally impacted if they were not located downstream of a waste release site and were not within one kilometer of a hazardous waste disposal area.

Drainage Unit	Stream	Site Name	Contaminant History
Upper Three Runs	Tinker Creek	TC	Minimal
	Mill Creek	MC	Non-radiological
	McQueen's Branch	MQ1	Mixed
		MQ2	Radiological
	Crouch's Branch	CB	Mixed
	Tim's Branch	TB	Non-radiological
	Upper Three Runs	UTR1	Minimal
		UTR2	Non-radiological
		UTR3	Non-radiological
Pen's Branch	Indian Grave Branch	IGB	Mixed
	Pen's Branch	PB1	Non-radiological
		PB2	Non-radiological
		PB3	Radiological
		PB4	Radiological
Steel Creek	Meyer's Branch	MB1	Minimal
		MB2	Minimal
	Steel Creek	SC1	Mixed
		SC2	Radiological
		SC3	Radiological

Table 4.2. Acoustic indices, their definitions, and relevant parameters used for this study. Further details on the calculation of these acoustic indices can be found in the Acoustic Index User's Guide (Bradfer-Lawrence et al., 2024).

Acoustic Index	Definition	Parameters
Bioacoustic Index (BI)	A product of the number of occupied, 1 kHz frequency bands and their amplitudes, relative to the quietest band. Initially designed to reflect bird diversity and/or abundance (Boelman et al., 2007).	Frequency: 2 – 8 kHz
Acoustic Complexity Index (ACI)	The mean relative difference in amplitude between five second time intervals, summed across frequency bands (Pieretti et al., 2011). Sensitive to variable amplitudes.	Frequency: 0.5-8 kHz
Acoustic Diversity Index (ADI)	The distribution of sound energy, measured by determining the proportion of cells above an amplitude threshold within frequency bands and then calculating the Shannon Diversity Index across the bands (Villanueva-Rivera et al., 2011).	Frequency: 0-10 kHz Threshold: -60 dB fs
Event Count Index (EVN)	The number of distinct acoustic events, defined as instances when a sound crosses an amplitude threshold in a noise-reduced spectrum (Towsey, 2018).	Threshold: 3 dB

Boelman, N. T., Asner, G. P., Hart, P. J. & Martin, R. E. 2007. Multi-trophic invasion resistance in Hawaii: bioacoustics, field surveys, and airborne remote sensing. *Ecological Applications,* 17**,** 2137-2144. https://doi.org/10.1890/07-0004.1

Bradfer-Lawrence, T., Duthie, B., Abrahams, C., Adam, M., Barnett, R. J., Beeston, A., Darby, J., Dell, B., Gardner, N., Gasc, A., Heath, B., Howells, N., Janson, M., Kyoseva, M.-V., Luypaert, T., Metcalf, O. C., Nousek-McGregor, A. E., Poznansky, F., Ross, S. R. P.-J., Sethi, S., Smyth, S., Waddell, E. & Froidevaux, J. S. P. 2024. The acoustic index user's guide: a practical manual for defining, generating, and understanding current and future acoustic indices. *Methods in Ecology and Evolution*. doi.org/10.1111/2041-210X.14357

Pieretti, N., Farina, A. & Morri, D. 2011. A new methodology to infer the singing activity of an avian community: the Acoustic Complexity Index (ACI). *Ecological Indicators,* 11**,** 868-873.

Towsey, M. 2018. The calculation of acoustic indices derived from long-duration recordings of the natural environment. QUT Ecoacoustics Research Group.

Villanueva-Rivera, L. J., Pijanowski, B. C., Doucette, J. & Pekin, B. 2011. A primer of acoustic analysis for landscape ecologists. *Landscape Ecology,* 26**,** 1233-1246.

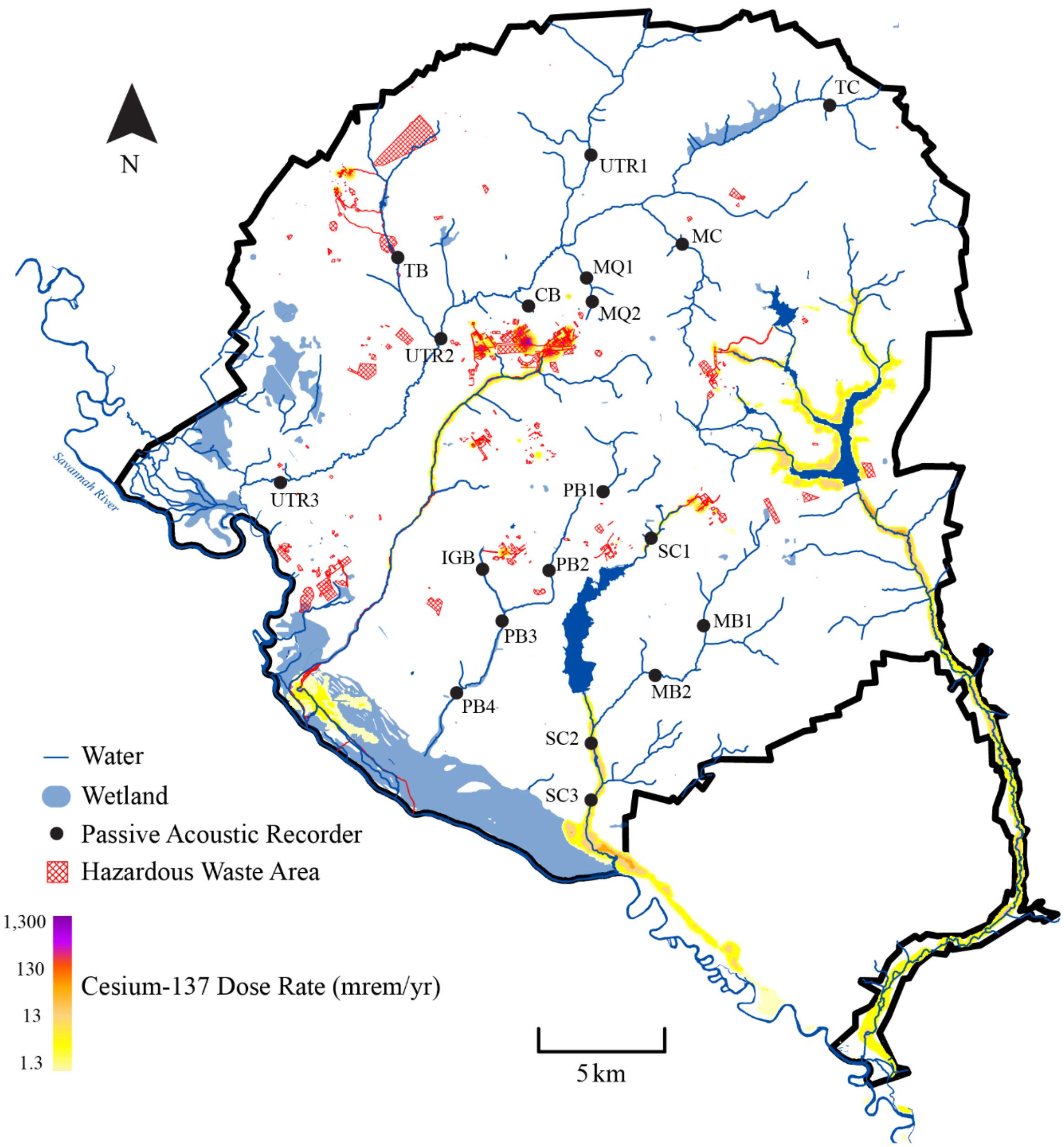

Figure 4.1. Locations of nineteen long-term monitoring sites across three interoperable units on the Savannah River Site. See Table 4.1 for the contaminant histories at each of the nineteen sites.

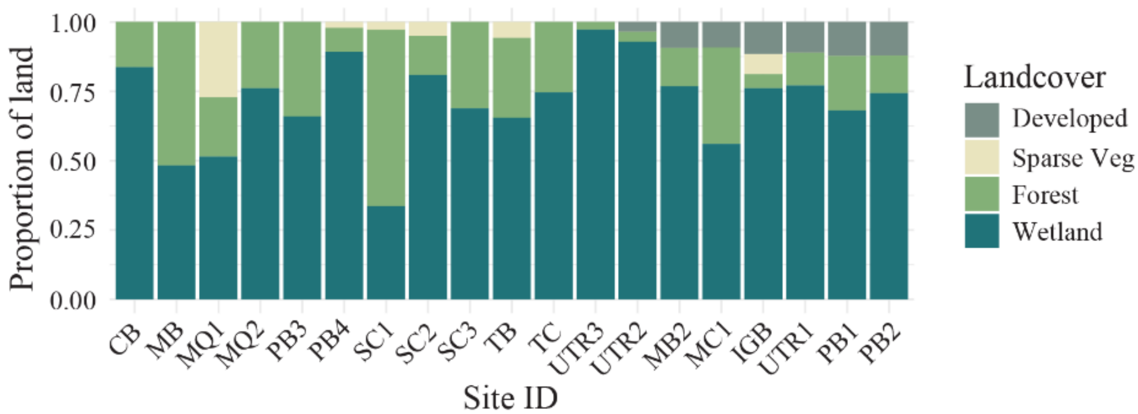

Figure 4.2. Proportion of each landcover type within 200m of each passive acoustic recorder on the Savannah River Site.

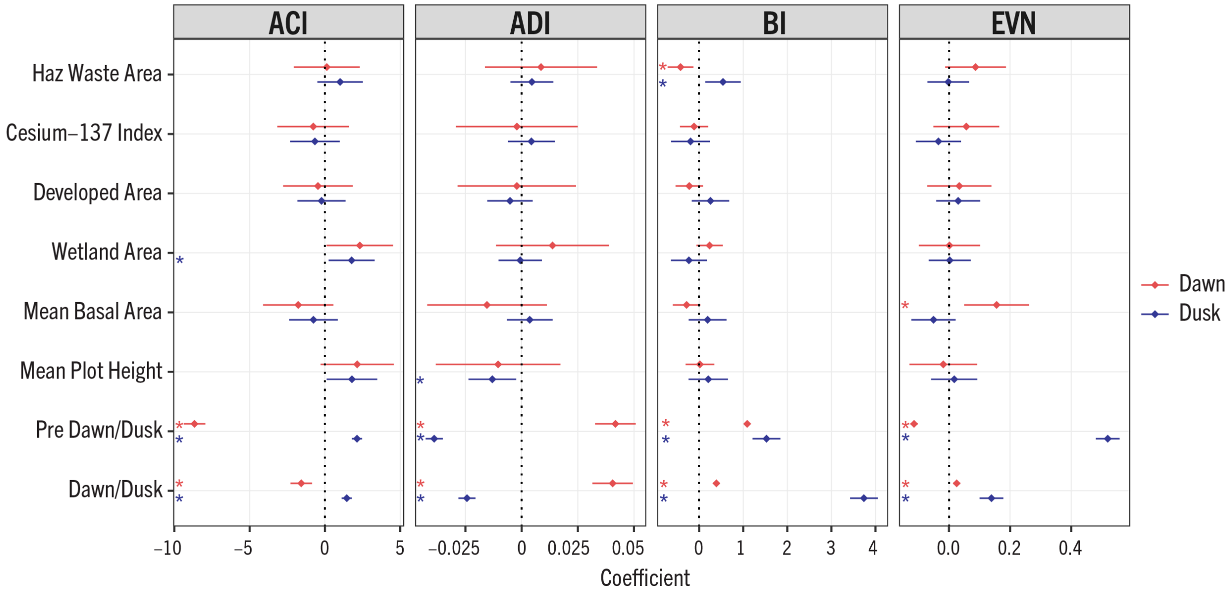

Figure 4.3. Estimated coefficients, with 95% confidence intervals, of the fixed effects included in linear mixed effects models evaluating contamination, landcover, and time period as predictors of four acoustic indices. The acoustic complexity index (ACI), acoustic diversity index (ADI), bioacoustic index (BI) and event count index (EVN) were derived from dawn and dusk soundscape recordings collected from nineteen locations on the Savannah River Site. The pre-dawn/dusk and dawn/dusk time categories are compared to the post-dawn/dusk reference level. Asterisks indicate effects where $p < 0.05$.

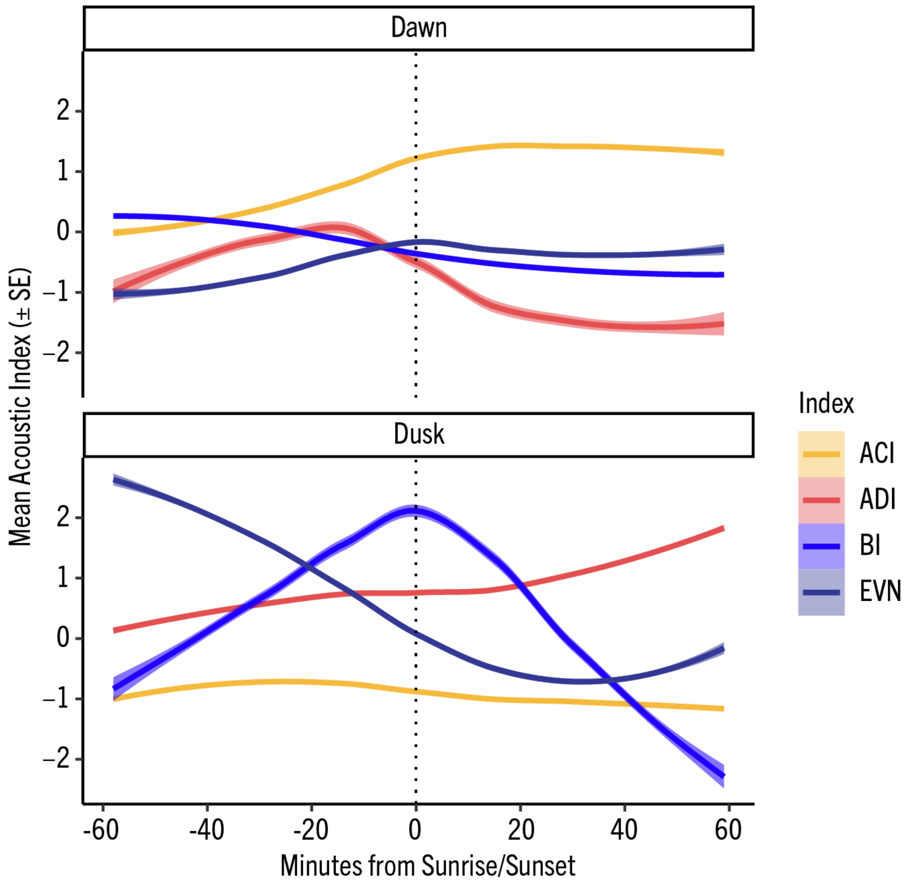

Figure 4.4. The mean acoustic indices for each minute of the dawn and dusk recordings across nineteen riparian monitoring locations on the Savannah River Site. Raw index values were standardized to a mean of zero and standard deviation of one for prior to plotting. The vertical dotted line represents sunrise and sunset.

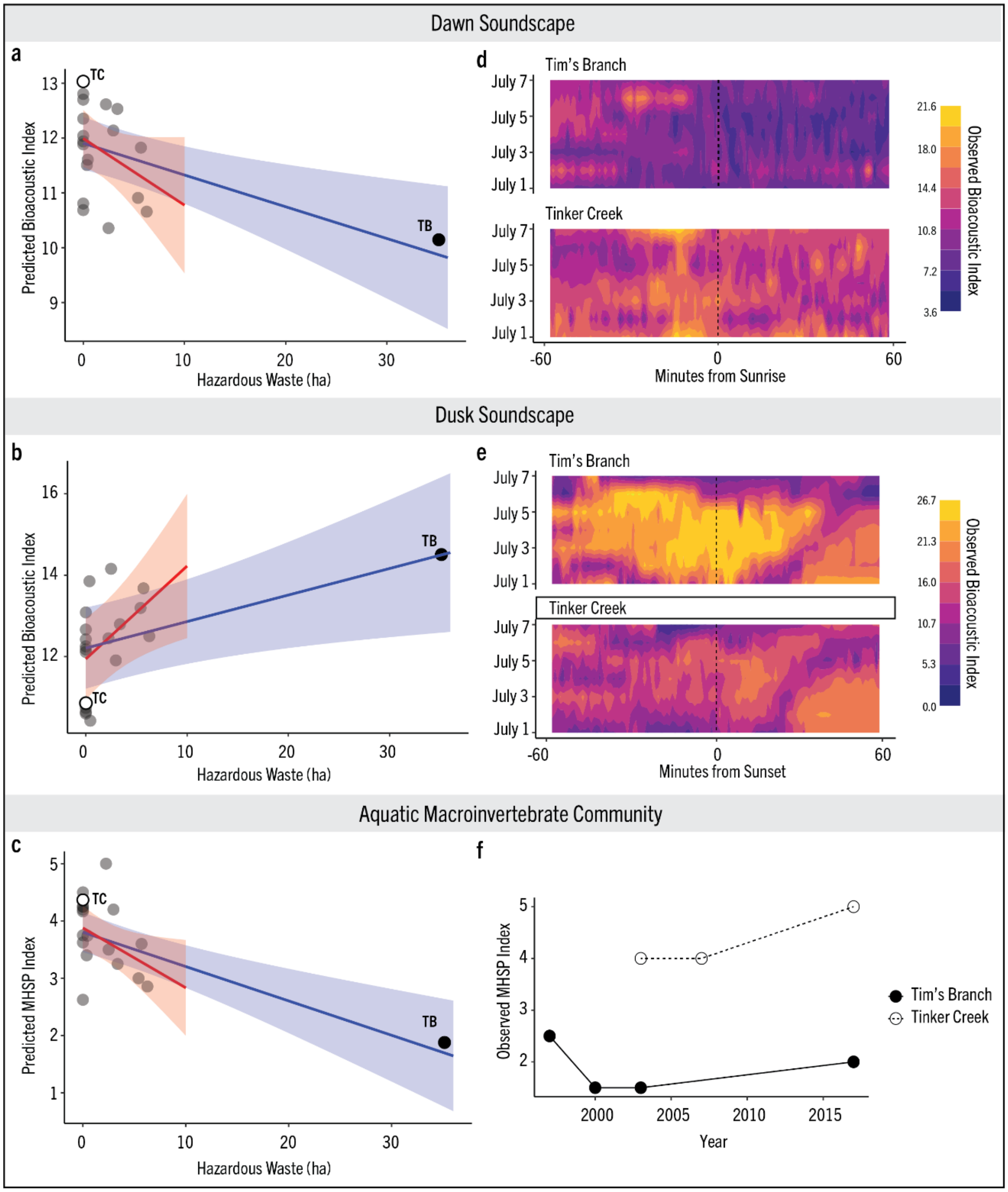

Figure 4.5. Predicted effect of hazardous waste disposal area, with 95% confidence intervals, on the **(a)** dawn bioacoustic index, **(b)** dusk bioacoustic index, and **(c)** aquatic macroinvertebrate multi-habitat sampling protocol (MHSP) index. Blue lines represent LMER models fit to the full dataset, whereas red lines represent LMER models fit to a subset of data excluding Tim's Branch (TB). Points represent average, observed index values for each of the nineteen sites. **(d)** Variation in observed dawn bioacoustic index and **(e)** dusk bioacoustic index over the course of each two-hour sampling period from a subset of recordings (July 1 to July 7) at TB and Tinker Creek (TC). **(f)** Observed MHSP index values from long-term monitoring at TB and TC (Paller et al. 2018).

CHAPTER 5

SUMMARY AND CONCLUSIONS

This thesis investigated the incorporation of waste-derived metals, metalloids, and radionuclides into multiple ecological processes relevant to passerine bird communities in contaminated habitat. In Chapter 2, we conducted a community-scale study of waste-derived element transfer to passerine birds inhabiting sites designated for long-term, *in situ* remediation of coal combustion residuals and nuclear fission products. Our multi-species approach allowed us to investigate whether species traits predicted variation in contaminant burdens. We found that passerines at legacy waste sites accumulated higher concentrations of selenium, mercury, arsenic, and cesium-137 compared to those at reference sites. Selenium and mercury uptake was mediated by dietary category, whereas arsenic and cesium-137 uptake was mediated by degree of terrestriality.

In Chapter 3, we investigated whether contaminants derived from coal combustion residuals and nuclear fission products altered the dynamics of haemosporidian blood parasites, dipteran vectors, and avian hosts in riparian and wetland habitats. We evaluated whether contaminants predicted the likelihood of single and coinfections by *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* within passerines and whether the likelihood of *Plasmodium* infection in *Culex* vectors differed with the presence of site-level contamination. We found that higher concentrations of Se in the blood of birds were associated with a lower likelihood of *Leucocytozoon* infection in birds, but contaminants did not increase the likelihood of parasite infection in birds or in *Culex* vectors.

In Chapter 4, we evaluated shifts in dawn and dusk chorus activity across contaminated landscapes using passive acoustic monitoring. We evaluated whether four acoustic indices varied with habitat type, structure, and extent of radiological and non-radiological waste across nineteen monitoring locations on the Savannah River Site. We found that the dawn bioacoustic index was significantly lower at sites affected by greater areas of non-radiological waste, indicating that the soundscapes of these contaminated sites are characterized by a lower intensity of biological sound produced by birds and other acoustically active organisms.

This thesis demonstrates the nuanced ecological impacts of legacy waste on wildlife communities. It addresses gaps in our understanding of the behavioral correlates of contaminant uptake among passerines, thereby providing insight into the mobility of waste-derived elements in ecosystems. It highlights the varied and dose-dependent effects of excess trace element concentrations on wildlife disease dynamics on contaminated landscapes. Finally, it demonstrates the utility of passive acoustic monitoring to detect contaminant impacts on the production of acoustic energy in ecosystems. Collectively, these three studies provide an ecological risk assessment that spans multiple ecological scales and offers new perspectives on biomonitoring, habitat management, and conservation strategies for contaminated lands.

APPENDIX A

CHAPTER 2 SUPPLEMENTARY INFORMATION

Supplemental tables provide additional information on heavy metals and metalloids measured in passerine blood samples collected from the Savannah River Site in 2023. Table A.1 reports percent recoveries of trace elements in certified reference material for quality control purposes. Table A.2 reports average trace element concentrations for common species captured at coal combustion residual and reference sites.

Table A.1. Average percent recoveries and standard deviations of trace elements in certified reference material (TORT-3 and DOLT-5) for ICP-MS and DMA.

Element	Mean Recovery (%)	Std. Dev (%)
Zn	95.9	7.7
Cu	89.3	5.6
Pb	107.2	21.3
Se	119.7	20.2
As	115.3	15.1
Hg	97.4	10.8

Table A.2. Mean blood trace element concentrations (ppm *dw*) and standard errors in passerines species captured at both CCR and Reference sites, excluding species where n < 4.

		ICP-MS (ppm *dw*)						DMA (ppm *dw*)	
Species	Site	N	Zn	Cu	Pb	As	Se	N	Hg
Agelaius phoeniceus	Ref	2	23.37 ± 9.03	1.34 ± 0.54	0.15 ± 0.19	0.02 ± 0.00	1.45 ± 1.36		
	CCR	4	16.61 ± 1.96	1.23 ± 0.22	0.01 ± 0.01	0.02 ± 0.00	4.84 ± 1.52		
Baeolophus bicolor	Ref	7	21.09 ± 1.36	0.47 ± 0.17	0.01 ± 0.00	0.02 ± 0.00	4.20 ± 0.87	7	0.22 ± 0.12
	CCR	17	20.95 ± 3.23	0.51 ± 0.15	0.01 ± 0.00	0.02 ± 0.01	5.57 ± 2.12	11	0.22 ± 0.06
Cardinalis cardinalis	Ref	18	20.54 ± 2.21	1.25 ± 0.18	0.02 ± 0.01	0.02 ± 0.01	3.48 ± 1.41	17	0.25 ± 0.11
	CCR	11	21.21 ± 1.84	1.20 ± 0.15	0.02 ± 0.02	0.03 ± 0.02	2.61 ± 1.31	9	0.19 ± 0.12
Empidonax virescens	Ref	2	21.33 ± 3.35	1.31 ± 0.22	0.01 ± 0.00	0.02 ± 0.00	5.22 ± 1.58		
	CCR	4	22.05 ± 1.89	1.49 ± 0.33	0.02 ± 0.01	0.03 ± 0.02	5.85 ± 1.80		
Geothylpis trichas	Ref	17	18.94 ± 3.83	1.25 ± 0.26	0.02 ± 0.03	0.03 ± 0.02	3.92 ± 0.87	5	0.55 ± 0.32
	CCR	15	16.91 ± 2.17	1.24 ± 0.19	0.02 ± 0.02	0.03 ± 0.02	4.52 ± 1.17	2	0.34 ± 0.11
Melospiza georgiana	Ref	7	22.84 ± 3.47	1.49 ± 0.46	0.22 ± 0.34	0.03 ± 0.04	5.35 ± 1.20		
	CCR	2	20.90 ± 0.01	1.06 ± 0.09	0.01 ± 0.00	0.02 ± 0.00	5.19 ± 1.67		
Mniotilta varia	Ref	2	21.09 ± 4.94	0.94 ± 0.13	0.01 ± 0.00	0.02 ± 0.00	6.59 ± 1.85		
	CCR	7	18.05 ± 2.24	1.10 ± 0.19	0.01 ± 0.00	0.03 ± 0.02	5.99 ± 1.60		
Myiarchus crinitus	Ref	8	17.29 ± 1.27	1.05 ± 0.22	0.01 ± 0.00	0.02 ± 0.00	6.82 ± 2.21	2	0.38 ± 0.05
	CCR	7	16.92 ± 1.74	1.10 ± 0.12	0.01 ± 0.00	0.02 ± 0.00	4.42 ± 0.94	7	0.93 ± 0.23
Parkesia motacilla	Ref	6	18.79 ± 2.90	1.42 ± 0.25	0.06 ± 0.05	0.06 ± 0.06	7.96 ± 3.68	3	1.20 ± 0.56
	CCR	5	17.50 ± 3.71	1.37 ± 0.27	0.13 ± 0.19	0.03 ± 0.02	11.22 ± 3.96	3	2.35 ± 1.01
Picoides pubescens	Ref	5	20.10 ± 1.87	1.47 ± 0.12	0.01 ± 0.00	0.02 ± 0.00	2.64 ± 0.78	4	0.12 ± 0.05
	CCR	4	22.39 ± 1.92	1.35 ± 0.12	0.01 ± 0.01	0.02 ± 0.00	3.54 ± 0.45	4	0.22 ± 0.16
Piranga rubra	Ref	7	20.35 ± 1.07	1.05 ± 0.19	0.01 ± 0.01	0.03 ± 0.02	4.22 ± 1.33	7	0.49 ± 0.32
	CCR	6	20.54 ± 4.30	1.09 ± 0.26	0.01 ± 0.00	0.05 ± 0.04	7.56 ± 2.26	4	0.61 ± 0.31
Poecile carolinensis	Ref	8	11.28 ± 2.51	0.51 ± 0.19	0.01 ± 0.00	0.02 ± 0.00	3.53 ± 1.08		
	CCR	5	11.60 ± 0.56	0.42 ± 0.07	0.02 ± 0.01	0.03 ± 0.01	6.37 ± 3.61		
Quiscalus quiscula	Ref	2	18.43 ± 1.70	1.13 ± 0.19	0.27 ± 0.23	0.02 ± 0.00	3.60 ± 0.30		
	CCR	8	20.29 ± 2.07	1.51 ± 0.32	0.11 ± 0.16	0.06 ± 0.05	6.56 ± 1.41		
Setophaga americana	Ref	2	19.16 ± 2.35	0.90 ± 0.22	0.01 ± 0.00	0.02 ± 0.00	4.13 ± 0.60		
	CCR	3	15.45 ± 0.66	0.94 ± 0.09	0.01 ± 0.00	0.02 ± 0.00	4.91 ± 1.82		
Setophaga citrina	Ref	7	18.33 ± 2.92	1.21 ± 0.21	0.01 ± 0.00	0.02 ± 0.00	5.10 ± 1.00		
	CCR	7	17.59 ± 1.51	1.20 ± 0.16	0.58 ± 1.49	0.03 ± 0.02	4.73 ± 1.57		
Setophaga pinus	Ref	4	18.50 ± 0.61	0.98 ± 0.25	0.01 ± 0.00	0.02 ± 0.00	5.12 ± 0.99		
	CCR	6	17.92 ± 2.71	1.00 ± 0.24	0.01 ± 0.00	0.02 ± 0.00	3.50 ± 0.80		
Thryothorus ludovicianus	Ref	17	25.28 ± 3.54	0.18 ± 0.07	0.01 ± 0.00	0.03 ± 0.02	4.35 ± 0.56	14	1.34 ± 0.55
	CCR	17	23.82 ± 2.43	0.16 ± 0.04	0.01 ± 0.00	0.02 ± 0.02	4.76 ± 1.10	9	1.41 ± 0.71
Vireo griseus	Ref	29	23.29 ± 3.69	1.61 ± 0.29	0.01 ± 0.00	0.02 ± 0.00	4.96 ± 1.75	3	0.71 ± 0.15
	CCR	26	22.10 ± 3.61	1.73 ± 0.32	0.01 ± 0.01	0.02 ± 0.01	5.55 ± 1.72	6	0.40 ± 0.09
Vireo olivaceus	Ref	12	20.03 ± 5.78	1.23 ± 0.34	0.01 ± 0.00	0.02 ± 0.01	7.32 ± 4.92	6	0.37 ± 0.12
	CCR	6	23.05 ± 2.83	1.49 ± 0.29	0.01 ± 0.00	0.02 ± 0.00	4.98 ± 2.08	2	0.52 ± 0.05

APPENDIX B

CHAPTER 4 SUPPLEMENTARY INFORMATION

Supplemental tables provide additional information on covariates of acoustic index values derived from midsummer dawn and dusk recordings collected from the Savannah River Site in 2024. Table B.1 and Table B.2 report results of linear mixed effects models for each of the four acoustic indices at dawn and dusk, respectively. Table B.3 reports results of linear mixed effects models for the historical biotic indices derived from macroinvertebrate and fish communities.

Table B.1. Results of linear mixed effects models showingthe relationship between each of the four acoustic indices derived from dawn soundscapes and predictor variables quantifying extent of contamination, vegetation structure, and time period. Predictors were standardized to a mean of zero and standard deviation of one. Significant predictors (α < 0.05) are indicated in bold.

Bioacoustic Index

Response	Fixed Effects	coef	std err	t value	p value
BI	Intercept	11.22	0.24	47.04	<0.001
	hazardous waste area	**-0.42**	**0.15**	**-2.82**	**0.01**
	cesium-137 index	-0.12	0.16	-0.71	0.49
	wetland area	0.24	0.15	1.57	0.13
	developed area	-0.22	0.16	-1.42	0.17
	mean plot height	0.02	0.17	0.11	0.91
	mean basal area	-0.29	0.16	-1.79	0.09
	period: dawn	**0.39**	**0.03**	**11.73**	**<0.001**
	period: pre-dawn	**1.09**	**0.03**	**32.50**	**<0.001**

Random Effects	variance	std dev
minute	0.02	0.12
date	1.29	1.13
site	0.39	0.63
residual	4.42	2.10

R^2	
marginal	0.09
conditional	0.34

Acoustic Diversity Index

Response	Fixed Effects	coef	std err	t value	p value
log(max(ADI + 1e-4) -ADI)	Intercept	-4.29	0.19	-22.37	<0.001
	hazardous waste area	-0.04	0.15	-0.27	0.54
	cesium-137 index	-0.10	0.16	-0.60	0.76
	wetland area	0.03	0.15	0.18	0.43
	developed area	0.11	0.16	0.68	0.59
	mean plot height	0.50	0.17	2.95	0.52
	mean basal area	-0.02	0.16	-0.15	0.15
	period: dawn	**-0.64**	**0.07**	**-8.75**	**<0.001**
	period: pre-dawn	**0.18**	**0.07**	**6.25**	**<0.001**

Random Effects	variance	std dev
minute	0.12	0.35
date	0.27	0.52
site	0.35	0.59
residual	1.01	1.00

R^2	
marginal	0.08
conditional	0.47

Acoustic Complexity Index

Response	Fixed Effects	coef	std err	t value	p value
	Intercept	643.58	2.38	270.92	0.000
	hazardous waste area	0.13	1.12	0.11	0.91
	cesium-137 index	-0.77	1.21	-0.64	0.53
	wetland area	2.32	1.13	2.06	0.05
ACI	developed area	-0.46	1.18	-0.39	0.70
	mean plot height	2.14	1.24	1.73	0.10
	mean basal area	-1.77	1.19	-1.49	0.15
	period: dawn	**-1.57**	**0.36**	**-4.31**	**0.000**
	period: pre-dawn	**-8.66**	**0.37**	**-23.62**	**0.000**

	Random effects	variance	std dev
	minute	2.28	1.51
	date	141.72	11.90
	site	21.79	4.67
	residual	207.16	14.39

R^2	
marginal	0.06
conditional	0.48

Event Count Index

Response	Fixed Effects	coef	std err	t value	p value
	Intercept	-2.04	0.22	-9.29	<0.001
	hazardous waste area	0.45	0.22	2.03	0.06
	cesium-137 index	0.19	0.24	0.80	0.43
	wetland area	0.13	0.22	0.58	0.57
log(EVN + 1e-4)	developed area	0.09	0.23	0.39	0.70
	mean plot height	-0.05	0.24	-0.20	0.84
	mean basal area	0.36	0.23	1.52	0.14
	period: dawn	**-0.04**	**0.02**	**2.04**	**0.04**
	period: pre-dawn	**-0.46**	**0.02**	**-24.62**	**<0.001**

	Random Effects	variance	std dev
	minute	0.00	0.05
	date	0.19	0.44
	site	0.84	0.92
	residual	3.14	1.77

R^2	
marginal	0.09
conditional	0.32

Table B.2. Results of linear mixed effects models showing the relationship between each of the four acoustic indices derived from dusk soundscapes and predictor variables quantifying extent of contamination, vegetation structure, and time period. Predictors were standardized to a mean of zero and standard deviation of one. Significant predictors (α < 0.05) are indicated in bold.

Bioacoustic Index

Response	Fixed Effects	coef	std err	t value	p value
	Intercept	10.66	0.51	20.93	<0.001
	hazardous waste area	**0.54**	**0.21**	**2.64**	**0.02**
	cesium-137 index	-0.20	0.22	-0.88	0.39
	wetland area	-0.23	0.21	-1.12	0.28
BI	developed area	0.26	0.22	1.19	0.25
	mean plot height	0.21	0.23	0.91	0.38
	mean basal area	0.19	0.22	0.88	0.39
	period: dusk	**3.74**	**0.16**	**23.38**	**<0.001**
	period: pre-dusk	**1.53**	**0.16**	**9.50**	**<0.001**

	Random Effects	variance	std dev
	minute	0.48	0.69
	date	7.49	2.74
	site	0.73	0.86
	residual	17.32	4.16

R^2	
marginal	0.10
conditiona l	0.40

Acoustic Diversity Index

Response	Fixed Effects	coef	std err	t value	p value
log(max(ADI + 1e-4) - ADI)	Intercept	-4.93	0.19	-25.70	<0.001
	hazardous waste area	-0.04	0.15	-0.27	0.79
	cesium-137 index	-0.10	0.16	-0.60	0.55
	wetland area	0.03	0.15	-0.18	0.86
	developed area	0.11	0.16	0.68	0.50
	mean plot height	**0.50**	**0.17**	**2.95**	**0.008**
	mean basal area	-0.02	0.16	-0.15	0.88
	period: dusk	**0.64**	**0.07**	**8.75**	**<0.001**
	period: pre-dusk	**0.83**	**0.07**	**11.14**	**<0.001**

	Random Effects	variance	std dev
	minute	0.10	0.32
	date	0.47	0.68
	site	0.40	0.63
	residual	2.67	1.63

R^2	
marginal	0.09
conditional	0.33

Acoustic Complexity Index

Response	Fixed Effects	coef	std err	t value	p value
ACI	Intercept	625.06	2.38	262.26	<0.001
	hazardous waste area	1.01	0.77	1.31	0.21
	cesium-137 index	-0.66	0.84	-0.79	0.44
	wetland area	**1.77**	**0.78**	**2.28**	**0.03**
	developed area	-0.23	0.81	-0.28	0.78
	mean plot height	1.79	0.86	2.10	0.05
	mean basal area	-0.76	0.82	-0.93	0.36
	period: dusk	**1.45**	**0.17**	**8.40**	**<0.001**
	period: pre-dusk	**2.13**	**0.17**	**12.27**	**<0.001**

	Random effects	variance	std dev
	minute	0.28	0.52
	date	184.35	13.58
	site	10.35	3.21
	residual	213.78	14.62

R^2	
marginal	0.02
conditional	0.49

Event Count Index

Response	Fixed Effects	coef	std err	t value	p value
log(EVN + 1e-4)	Intercept	-2.24	0.20	-10.99	<0.001
	hazardous waste area	0.04	0.09	0.41	0.69
	cesium-137 index	-0.10	0.10	-0.99	0.34
	wetland area	0.00	0.09	0.02	0.98
	developed area	0.03	0.10	0.36	0.73
	mean plot height	-0.02	0.10	-0.15	0.89
	mean basal area	-0.14	0.10	-1.40	0.18
	period: dusk	**-0.07**	**0.04**	**-1.61**	**0.11**
	period: pre-dusk	**0.86**	**0.04**	**20.17**	**<0.001**

	Random Effects	variance	std dev
	minute	0.03	0.17
	date	1.19	1.09
	site	0.15	0.38
	residual	5.32	2.31

R^2	
marginal	0.03
conditional	0.23

Table B.3. Results of linear mixed effects models evaluating hazardous waste disposal area and cesium-137 index as predictors of the multi-habitat sampling protocol index (MHSP) and the index of biotic integrity (IBI). The MHSP and IBI were derived from long-term monitoring of aquatic macroinvertebrate and fish assemblages, respectively, at nineteen long-term monitoring locations on the Savannah River Site. Predictors were standardized to a mean of zero and standard deviation of one. Significant predictors ($\alpha < 0.05$) are indicated in bold.

Multi-Habitat Sampling Protocol Index

Response	Fixed Effects	coef	std err	t value	p value
	Intercept	3.58	0.16	21.74	<0.001
MHSP	**hazardous waste area**	**-0.47**	**0.11**	**-4.23**	**<0.001**
	cesium-137 index	-0.12	0.11	-1.09	0.28

	Random Effects	variance	std dev
	site	0.03	0.18
	year	0.07	0.27
	residual	0.76	0.87

R^2	
marginal	0.21
conditional	0.31

Index of Biotic Integrity

Response	Fixed Effects	coef	std err	t value	p value
	Intercept	40.27	1.42	28.45	<0.001
IBI	hazardous waste area	-2.46	1.40	-1.76	0.10
	cesium-137 index	-1.98	1.36	-1.46	0.16

	Random Effects	variance	std dev
	site	29.35	5.42
	year	0.00	0.00
	residual	34.45	5.87

R^2	
marginal	0.12
conditional	0.53