Monitoring Potential Cadmium Levels in Avian Tissues Associated with the Savannah Harbor Expansion Project: Year 2 of Monitoring Annual Report

O.E. Rhodes, Jr.[1], S.B. Wilde[2], J.C. Seaman[1] and A.L. Bryan, Jr.[1]

[1]University of Georgia's Savannah River Ecology Laboratory, Aiken, SC
rhodes@srel.uga.edu (803-725-8191: OER); seaman@srel.uga.edu (803-725-0977);
lbryan@srel.uga.edu (803-725-2907:ALB) [2]University of Georgia, Warnell School of Forestry and Natural Resources, Athens, GA swilde@uga.edu (706-542-3346)

INTRODUCTION

The Savannah Harbor Expansion Project (SHEP) involves dredging the Savannah River and will eventually result in deposition of sediment containing potentially high concentrations of Cadmium (Cd) into dredged material containment areas (DMCAs) near Savannah. Cadmium is a toxic metal with both natural and anthropogenic sources and no known nutritional value (Wayland and Scheuhammer 2011). Effects of toxic concentrations of Cd on birds range from organ damage to behavioral changes. The SHEP DMCAs contain upland and aquatic habitats and are bounded by salt marsh and riverine habitats, which tend to attract a wide range of avifauna. Dredged material deposition may be a potential cadmium exposure route for birds attracted to these impoundments. As part of the monitoring program to determine potential environmental impacts associated with the SHEP, we examined Cd uptake by avian species associated with containment areas in 2014/2015, a sampling period prior to deposition of dredged materials containing the Cd. Inner harbor dredging did not occur in 2015/2016 (Year 2) and samples collected in this period are included as an additional year of pre-construction monitoring.

The strategy to examine potential Cd risk to avifauna using the DMCAs was to document Cd uptake in a set of sentinel avian species. These sentinel avian species, which were monitored during two seasons (summer and winter), were determined by USACE and the US Fish and Wildlife Service (USFWS) and based on long-term monitoring of avian occurrences at the DMCAs. Suites of available sentinel species were influenced by the hydrologic condition (wet vs dry) of the DMCAs. In the second year of this monitoring, as in the first year, we attempted to monitor all sentinel species to provide a baseline for future monitoring. Monitoring in subsequent years, during potential Cd deposition and bioavailability, will likely focus on a reduced number of sentinel species, but with a greater number of samples per species.

The primary approach to assess Cd risk, as requested in the SOW, was to analyze blood for Cd from the sentinel resident and migratory species because concentrations of Cd in blood are indicative of recent uptake in adult birds. Cadmium can be removed from blood as quickly as 48-hours (Sell 1975). The use of blood from live-captured birds for metals analysis is a nonlethal technique that has little impact on avian populations, but that allows for concomitant assessments of health. After the initial year of sampling, we determined that certain species

(e.g.; Mottled Ducks, Northern Shovelers, American Avocets, Mourning Doves) could not be live-captured effectively and, after discussions with USACE, acquired permits for lethal take of selected species. In addition to the blood samples collected from the lethal take, this method allowed us to examine blood concentrations relative to other tissues of interest (e.g., kidney and liver). As optional monitoring, we opportunistically collected avian prey base samples (invertebrates; e.g., aquatic and terrestrial insects, and snails) from the DMCAs and analyzed them for Cd to provide insights into potential transmission routes of Cd into sentinel and other non-sentinel wildlife.

Our goal in the second year of this project was to continue capturing sentinel avian species and collecting blood samples for chemical (primarily Cd) analyses during the Summer (2015) and Winter (2015/2016) seasons, as well as the concentrations in associated tissues and potential prey base. Here we report our captures, lethal takes, and the contaminant findings of the avian blood and tissue analyses.

METHODS

Study Area

The DMCAs targeted for receiving the sediments potentially contaminated with Cd are 14A and 14B, so our avian collections were originally to be focused within and adjacent to these areas. They are bordered on the north by salt marsh and tidal creeks and to the south by the Savannah River. Dikes around the perimeter will maintain standing water within the DMCA, but the perimeter will still support shrubs and small trees, providing habitat for additional species (Figure 1).

However, construction activities at DMCA 14B during the first and second year of monitoring prevented us from accessing or collecting any samples from that area. In addition, the impacts of heavy rainfall and large equipment used on the DMCAs occasionally rendered several of the access roads impassable for short periods, which also influenced avian sampling. After discussions with USACE given (1) the lack of access to 14B, (2) the occasional lack of access to 14A due to poor road conditions, and (3) that this was a "control"/pre-Cd deposition year, we attempted to focus sampling effort on 14A and adjacent 13B but agreed to include captures from five DMCAs (12A, 13A, 13B, 14A, 14B) within the Savannah Harbor Navigation Project.

Avian Capture/Blood & Tissue Collection Methods

We employed multiple techniques for capturing DMCA avifauna. Most of the sentinel species (Table 1) were passerines and were captured with mist nets. These nets (30-mm mesh, 2.4m tall X 12m long) were typically deployed in cleared lanes either adjacent to shrubby habitat or through forested habitat during the morning/early afternoon period. Playback equipment broadcasting specific bird songs were often used to attract and capture specific species. Baited enclosure traps deployed to capture mourning doves were not effective in year 2 and we utilized

lethal take (shotgun) for this species. Black-necked stilts were captured by two methods. We employed nest traps to capture nesting adult stilts (in May), which involved flushing an attending parent off a nest with eggs and then covering the nest with a "funnel trap" made of light-weight fencing (1m tall, 1.2m diameter). After deploying the trap, the parent would return to the nest and enter the trap through the funnel to continue incubating the eggs and we would hand-grab the bird. A second method for the stilts, later in the summer, was to hand-capture and collect samples from pre-flight age stilt nestlings. In year 1 we had utilized a WCS NetBlaster to capture individual foraging stilts but the methods listed above were found to be more efficient and of less risk to the stilts.

We have had no success live-capturing three aquatic sentinel species: Mottled Ducks (summer species), Northern Shovelers (winter species), and American Avocets (winter species). We attempted using the NetBlaster to capture both duck species but the ducks either did not use shoreline habitats where the NetBlaster can function or were wary of the NetBlaster set-up. We also attempted baited (corn) enclosure/funnel traps with decoys which will occasionally capture many species of waterfowl, but these traps were not effective possibly due to both species being largely insectivorous. We received permission from USACE and scientific collecting permits from USFWS/SCDNR to collect these species lethally (e.g., shotgun). Unfortunately, we did not receive the USFWS permit until mid-summer of 2015, which prevented our collection of early summer season Mottled Ducks. Finally, Mourning Doves were successfully captured in walk-in traps in Year 1 but that method was unsuccessful in Year 2 for unknown reasons. We collected doves lethally in the late summer of Year 2.

Sentinel Species & Potential Alternative Species

The sentinel species collection SOW (provided by USACE) targeted collecting blood from a minimum of 10 individuals of each sentinel species during each season (summer vs winter). To the extent possible, the within-season collections were to be split between early and late periods: Summer – April & August; Winter – October/November & March/April. However, the availability of certain target species was influenced by existing hydrological conditions and/or the behavior of the species. As mentioned above, we had varied success capturing the project sentinel species. As a result of this, during each field season we banded and collected blood samples from some potential alternative avian species in case capturing the sentinel species proved problematic. In Year 2 these potential "alternate" species included Common Ground Doves and Rock Pigeons (for Mourning Doves), Northern Cardinals, and Swamp Sparrows. These species were often easier to capture than the sentinel species and we include their Cd concentrations in this report for comparison and additional background information.

<u>Avian Blood/Tissue Collections</u>

For all live-captures, we collected blood samples from either the jugular, brachial (wing) or femoral (leg) vein with a syringe. Volumes of blood collected ranged from < 0.05 ml to 0.80 ml,

depending on bird condition, size, and method (live vs lethal) of collection (we were permitted to collect blood volume NTE 1% of the bird's weight). Blood samples were archived (frozen) prior to chemical analysis. Captured individuals received appropriately-sized USGS aluminum leg bands prior to release to allow potential observations of marked birds and/or recaptures (and resampling) of known individuals. Resampling (blood) from recaptured birds, although not common, allows for between/among year comparisons. These blood collection/banding activities were conducted under SREL's bird banding permit (BBL permit # 22002).

As mentioned above, select species were collected by lethal methods (shotgun). In addition, a small number of birds (N=2) died during the handling process. These birds were immediately dissected to access the heart and blood was drawn from the organ with a syringe and archived as with the other blood live-capture collections. The carcasses were stored in ice and eventually frozen. After returning to SREL, kidney, liver, and gizzard contents were removed from the carcasses for analyses for contaminants and comparison to the blood concentrations. These activities were conducted under SREL's federal migratory bird scientific collecting permit (MB65214A-0) as well as state of South Carolina scientific collecting permits.

Optional nestling tissue collections (e.g., blackbird nestlings) did not occur in the second year of the project.

Optional Prey Base Collections

Preliminary collections of potential prey base (for avian species) samples within and/or adjacent to the DMCAs occurred again in Year 2. Collections of aquatic and terrestrial insects and other potential prey involved use of sweep nets and other devices. Seeds, fruits and Duckweed were collected by hand. Vegetation (leaves) of selected plants was collected to determine if they were the source of Cd for the insects. Due to the small size/mass of many potential prey items, we combined many items into composite samples prior to analyses.

Chemical Analyses

Biological samples (e.g., blood, tissues, prey) were analyzed for Cd as well as a suite of other metals (As, Cr, Cu, Ni, Pb, Se, Sr, Zn) at the University of Georgia's Savannah River Ecology Laboratory. Samples were digested in multiple batches of 12, which included two sample replicates, a reagent blank, and a certified standard reference material, following EPA method 3052. After digestion, trace element analyses were performed according to EPA method 6020, utilizing an ICP-MS NexION 300X, (PerkinElmer Inc., Toronto, Canada). Tissue concentrations of elements were reported in ppm (µg/g) wet weight for blood and ppm (µg/g) dry weight for avian tissues and potential prey items.

Sample-specific method detection limits (MDLs – Cd concentrations below these levels could not be accurately determined given the instrumentation, volumes of samples, and Cd concentrations within the samples) ranged from 0.00185 – 0.07166 Cd ppm wet weight for avian

blood, and was 0.013 µg/g dry weight for avian tissues and potential prey items. MDLs for avian blood and tissues were far below the Cd concentrations listed as potential levels resulting in toxic effects (see Discussion).

Concentrations of elements other than Cd in blood, avian tissues, and prey are documented in Appendices 1, 2, and 3.

RESULTS

Blood Concentrations

In the second year of sampling, 111 blood samples were collected from birds associated with the Savannah DMCAs, including 98 (total) from the 11 sentinel species and 13 from alternative species. Thirty-one of these samples (27.9%) were above the MDLs (0.00185 – 0.07166 ppm wet wt) for Cd (Table 2). The majority of these above MDL samples (28 of 31, ~ 90%) were collected during the Summer sampling period (April-August). The avian species with the highest percentage of >MDL Cd blood concentrations were "terrestrial" species: Brown Thrasher (89%), Mourning Dove (56%), and Northern Mockingbird (56%). Of the four "aquatic" sentinel species, Black-necked Stilts had the highest percentage (33%) of >MDL blood concentrations. The maximum blood Cd concentration observed in the second year of monitoring was 0.09 ppm (wet wt) in a Mourning Dove, far below the concentration (0.26 ppm wet wt.) associated with possible toxic effects for Cd (see Discussion).

Relative to species, Summer blood samples of thrashers averaged 0.0223 ppm Cd (n=9, SD=0.0104) whereas doves and mockingbirds (summer only) averaged 0.0222 (n=9, SD=0.0269) and 0.0069 ppm Cd (n=7, SD=0.0030), respectively (in these calculations, a <MDL concentration was replaced by ½ of its MDL).

A single bird (N. Mockingbird) banded in Year 1 was recaptured in Year 2. Its blood Cd concentration was < MDL in Year 1 and was 0.007 ppm Cd in Year 2.

Tissue Comparisons

We lethally collected 6 American Avocets, 4 Mottled Ducks, 9 Mourning Doves, 8 Northern Shovelers, and had two passerine netting mortalities (a Savannah Sparrow and a Song Sparrow) which allowed us to examine Cd concentrations among other tissues and gizzard contents. Kidney and liver are the primary storage organs for Cd, and we analyzed gizzard contents as an indicator of recent dietary intake Unfortunately, blood Cd concentrations of most of these collected birds were <MDL, which precluded comparisons of these tissues with blood levels for all species except Mourning Doves/Rock Pigeons (N=7). There was no significant correlation between blood and either gizzard content, kidney or liver concentrations (Spearman Rank Correlation, r_s = 0.0853 – 0.3929, Probs > $|r|$ = 0.2939 – 0.6445). Kidney concentrations were 2-

20 times higher than liver concentrations among all species (Table 3). None of the kidney and liver concentrations of Cd were greater than the concentrations reported to impact avian wildlife (see Discussion). Assessment of the relatedness of kidney and liver tissues varied by species (Table 4) although the tissues were significantly related overall (N=31, Spearman Rank, r_s = 0.7621, Prob > |*r*| = 0.0001). Gizzard contents averaged < 0.5 ppm Cd dry wt. and varied considerably among species (Table 3).

Potential Prey Base

Cadmium concentrations in potential prey items collected on the DMCAs were as highly variable in the second year of monitoring (Table 5), as they were in the first year. Larval potato beetles (Family Chrysonelidae) again had the highest average Cd concentration (> 24 ppm dry wt) among potential prey items. These larvae typically foraged on leaves of Tropical Soda Apple (*Solanum viarum*), which had a Cd concentration of ~17 ppm Cd dry wt. Duckweed (Lemnaceae), a small floating aquatic plant, had a Cd concentration of ~2.7 ppm whereas fruit of Blackberry (*Rubus* sp.) and Chinese Tallow averaged ~1.4 ppm Cd. Plant seeds and other insects all averaged < 1.0 ppm Cd.

DISCUSSION

Cadmium concentrations in birds vary widely according to ecosystem use, diet, age, and physiological status (Wayland and Scheuhammer 2011). Relative to ecosystem use, seabirds typically have > Cd than coastal birds, which typically have > Cd than freshwater wetland birds, which typically have > Cd than terrestrial birds. Dietary exposure to Cd tends to be greatest with insectivores, which includes several monitored seabird species such as albatrosses, petrels, and storm petrels (Wayland and Scheuhammer 2011), as opposed to piscivores or scavengers, but this varies. Cadmium does not "biomagnify" throughout trophic levels like some elements (e.g., mercury), but can biomagnify in lower trophic levels (e.g., water or sediment to lower invertebrates) (Eisler 1985, Scheuhammer 1987). Insects and their larvae, which are often more-closely associated with potentially-contaminated sediments, may provide the more likely pathway for Cd exposure to avifauna.

Within avian tissues, concentrations of Cd in kidney and liver reflect chronic exposure whereas blood concentrations reflect recent dietary exposure. The majority of Cd in birds is found in the kidney and liver, which typically accounts for 67-97% of the total body burden of Cd. Kidneys are the long-term storage organ for Cd, whereas liver concentrations are generally only 10-70% of kidney concentrations. Cadmium concentrations in blood are generally very low ($\leq$ 0.50 µg/g Cd wet wt). Age and diet are the two most important factors influencing Cd accumulation, with older/adult birds typically having considerably higher Cd concentrations than younger, developing birds.

Avian tissue concentrations of Cd associated with potential toxic effects (e.g.; organ damage, reduced breeding success, altered behaviors, etc.) are largely based on laboratory uptake studies

and vary widely among species. Kidney concentrations > 65 µg/g Cd (wet wt) are associated with a 50% likelihood of negative effects, although 100 µg/g Cd (wet wt) is a more liberal level of potential effects (Wayland and Scheuhammer 2011). Liver concentrations greater than 45-70 µg/g Cd (wet wt) and blood concentrations $\geq$ 0.26 µg/g Cd (wet wt) are suggested to be associated with toxic effects.

Concentrations of Cd in blood of avian sentinel species from the Savannah Harbor DMCAs in Year 2 remained generally low, with approximately 72% being below the MDL range of 0.00129-0.07166 µg/g Cd (wet wt), and no samples were greater than the concentration 0.26 µg/g Cd (wet wt) suggested to be associated with toxic effects. These results are similar to the findings in Year 1 (~71% < MDL; Rhodes *et al*. 2015). Also as in Year 1, more terrestrial birds than aquatic birds had concentrations of blood Cd > than the MDLs. This is contrary to expectations that birds utilizing aquatic habitats should have higher Cd concentrations (Wayland and Scheuhammer 2011). Analyses of a limited set of potential avian invertebrate prey types collected on-site in Year 2 again also documented highly variable Cd bioavailability in the existing DMCA environment. The composite potato beetle larvae sample had a high Cd concentration (27 ppm Cd dry wt) again in Year 2 (15 ppm Cd average in Year 1), as did Tropical Soda Apple leaves, an invasive species where the larvae were collected. Other terrestrial vegetation and fruit had Cd concentrations between 1-3 ppm Cd (dry wt). Most potential aquatic prey was relatively low in Cd, the exception being Duckweed, a small aquatic floating plant with a Cd concentration ~2.5 ppm. Variability in bioaccumulation of Cd by plants has been documented (Eisler 1985, Rodrigue *et al*. 2007). Brown Thrashers, which again had the highest occurrence of above-detection level Cd concentrations in blood, are typically insectivores and often specialize in beetles (Cavitt and Haas 2014). Thrashers are ground feeders that often feed by sweeping their bill through leaf litter to expose insects, so incidental sediment ingestion could also be a Cd exposure pathway. All thrasher blood samples came from the interior of DMCA 14A.

Analyses of potential within season (early vs. late) and between season (summer vs. winter) differences in blood Cd levels was limited by detection levels and sample sizes. So few of the blood samples were above the MDLs that statistical comparison within and between seasons were not feasible. General within-season comparisons of three summer sentinel species (stilts, thrashers, and mockingbirds) did not demonstrate differences in Cd concentrations or percentages of >MDL. Of note is that all of the late season stilt blood concentrations came from pre-flight juveniles and thus any Cd within their blood (2 of 3 were below MDLs) and/or tissues would originate from the DMCAs. Numbers and percentages of above MDL sample concentrations (all species) suggests greater exposure to Cd in the summer months, although general comparisons like this are confounded by a lack of similar species, and numbers of samples of similar species, between seasons. The greater percentage during the summer season may be linked to greater insect abundance and/or larvae emergence during the spring/summer months. These within and between season comparisons may be more informative in the future

sampling when the number of sentinel species are reduced to two per season and the number of individuals per species per season increases from a maximum of 10 to 30.

Permission was obtained to lethally-collect those species (Mottled Ducks, Northern Shovelers, American Avocets, and Mourning Doves) that had not been prone to live capture, although USFWS permits did not arrive until mid-summer. The late arrival of the permits precluded the collection of early season dove and Mottled Duck samples. Lethal collection of Northern Shovelers and Mourning Doves proved very effective in 2015/2016. Collection of avocets had proven difficult due to the specific habitats they utilize, typically areas where dredge material is actively being pumped into the DMCAs which are inaccessible to field personnel. Facility activities on the DMCAs in 2015/2016 (moving dredge pipe from 12A to 14B) created a situation where the avocets switched from their typical habitats to shoreline habitats and allowed collection by lethal methods.

Analyses of Cd concentrations among lethally-collected avifauna blood and tissue samples were not feasible due to < MDL Cd concentrations for avian blood. Concentrations of Cd in other tissues indicated accumulation primarily in kidneys, as expected. Liver concentrations were also all above detection limits, but considerably lower than kidney concentrations. Unlike Year 1, gizzard content samples (recent food) for all species were above the Cd MDL, which suggests elevated levels of Cd within both terrestrial and aquatic components of the DMCA system. This is also supported by the Cd concentrations found in blood samples from both aquatic and, primarily, terrestrial birds. As with the blood sampling effort, these findings will be enhanced by increased sample sizes and analysis of a greater number of potential prey items.

The findings of the two combined years of avian monitoring (Table 6) are similar to those of the individual year findings. For the pre-construction monitoring, terrestrial birds exhibited a higher percentage of > MDL blood Cd concentrations than aquatic species and, among terrestrial species, summer samples exhibited a higher percentage of >MDL blood concentrations than winter samples. Presumably the latter is due to greater insect availability as prey during this season.

Variation in capture/collection methods to obtain the blood samples for analysis resulted in varying levels of success among species. As mentioned previously, lethal collection (primarily in Year 2) proved effective for both duck species and doves. Success of lethal collections of avocets was strongly linked to water levels/pumping activities within the DMCA. Successful collection of avocets is primarily dependent upon their use of locations where we can shoot and retrieve the birds. Early season stilt captures were conducted with either a NetBlaster or nest trap (we have shifted almost entirely to the nest trap for greater efficiency) and late season stilt samples tends to be pre-flight nestlings which are literally chased down by an individual with a net. The remaining bird species are captured with mist nests, in combination with either play-back devices, decoys or both. Capture success among the terrestrial birds varied by species and season. We had greater success with three of the resident (both seasons) species (Brown

Thrasher, Eastern Towhee, and Northern Mockingbird) in the summer season, possibly due to their greater response to play-backs during the nesting season. However, overall capture rates for both thrashers and towhees were lower than desired (Table 6, also see discussion below). For Northern Cardinals, an alternate resident species, we had good capture rates in the winter season. For winter migrants, mist nets proved very effective at capturing adequate numbers of Savannah and Song Sparrows.

An inherent component of the initial years of avian monitoring was the determination of which set of sentinel species (both within and between habitats and seasons) would be appropriate for the focused sampling (~30 samples per each of two species per season) when the Cd-contaminated sediment is added to the DMCAs. The avian capture results through the initial two years of monitoring (Table 6) provide insights into what species might be feasible for such focused sampling. Cumulative within-season sample sizes of ~20 indicates we met the initial goal of 10 per species per season per year whereas sample sizes far below 20 indicate capture difficulty and suggest that the likelihood of focusing on these species is unlikely to result in attaining the desired goal of 30 samples in focused sampling. Possible exceptions to this are American Avocet and Mottled Duck if conditions are optimal for their use of the active DMCA and they are in locations suitable for lethal collection. Selection of the focal species will also be dependent upon the landscape/habitat availability in the area of the active DMCA as well as its hydrologic condition (wet or dry). Depending on these environmental conditions and subsequent presence of sentinel species on/near the active DMCA site, we would recommend Mourning Doves and Red-winged Blackbirds as summer season species and Northern Shovelers and Savannah Sparrows as winter season species based on our capture/collection efforts to date. If an aquatic sentinel species is preferred for the summer season, we would utilize the species (Black-necked Stilt or Mottled Duck) present in greatest numbers on the active DMCA, although attaining the desired sample size may prove to be more difficult.

SUMMARY

The majority of avian blood samples (~72%) collected from the Savannah Harbor DMCAs in the second year of monitoring was below the range of instrument detection levels (0.00129-0.07166 µg/g Cd wet wt). This finding is similar to those of Year 1. No blood sample was found to be above the level of concern for potential toxic effects. The majority of the above-MDL samples were collected during the summer months, possibly due to the greater availability of certain species of insects (a food source) during the period that bioaccumulate Cd. This trend is also apparent in the cumulative avian blood samples (Years 1 & 2) presented in Table 6, which also indicates capture success rates between seasons. Cadmium found in blood, gizzard contents (from lethal collections) and on-site potential prey indicate that Cd is currently bioavailable in the DMCA system and/or that these birds forage at nearby sites with bioavailable Cd. Analyses of kidney and liver tissues from lethally-collected avifauna on the DMCAs indicate that these species are accumulating Cd and that they are also likely accumulating Cd from other locations utilized in their history.

Lethal collections were employed to obtain blood/tissue samples from several species that had been difficult to capture by non-lethal methods (e.g.; traps, Net Blaster, etc.). This method may need to be expanded to obtain other difficult-to-obtain avian samples, pending acquisition of appropriate scientific collecting permits.

ACKNOWLEDGEMENTS

Chris Depkin, Meghan Oberkircher, Brigette Haram, Tabitha Phillips and Elizabeth Kurimo of the University of Georgia, and Chris Copella (USFWS) assisted with field collections. Katrina Ross, Brian Croft, Meghan Oberkircher and Angela Lindell of the UGA-SREL assisted with the analyses of the blood, tissue and prey base samples for Cd and other metals. We received administrative and logistic support and cooperation from many agencies/personnel associated with the Savannah Harbor Expansion Project, particularly the following USACE personnel: William Bailey, Ellie Covington, Nathan Dayan, Burton Moore, and Mary Richards. This project was funded by a grant from the U.S. Army Corps of Engineers (Cooperative Agreement W912HZ-14-2-0003) and received additional support from the Department of Energy, Office of Environmental Management under Award Number DE-FC09-07SR22506.

LITERATURE CITED

Cavitt, J.F., and C.A. Haas. 2014. Brown thrasher (*Toxostoma rufum*), The Birds of North America Online (A. Poole, ed.), Ithaca, Cornell Lab of Ornithology, retrieved from http://bna.birds.cornell.edu/bna/species/557.

Eisler, R. 1985. Cadmium hazards to fish, wildlife, and invertebrates: A synoptic review. U.S. Fish and Wildlife Service Biological Report 85(1.2), 46 pp.

Rhodes, O.E., S.B. Wilde, and A.L. Bryan. 2015. Monitoring potential cadmium levels in avian tissues associated with the Savannah Harbor Expansion Project: Year 1 of construction annual report. Annual report to the U.S.A.C.E., Savannah District. University of Georgia's Savannah River Ecology Laboratory, Aiken, SC.

Rodrigue, J., L. Champoux, D. Leclair, and J.-F. Duchense. 2007. Cadmium concentrations in tissues of willow ptarmigan (*Lagopus lagopus*) and rock ptarmigan (*Lagopus muta*) in Nunavik, northern Quebec. *Environmental Pollution* 147:642-647.

Scheuhammer, A.M. 1987. The chronic toxicity of aluminum, cadmium, mercury, and lead in birds: A review. *Environmental Pollution* 46:263-295.

Sell, J.L. 1975. Cadmium and the laying hen: Apparent absorption, tissue distribution and virtual absence of transfer to eggs. *Poultry Science* 54:1674-1678.

Wayland, M., and A.M. Scheuhammer. 2011. Cadmium in birds. Pp. 645-666 in *Environmental Contaminants in Biota: Interpreting Tissue Concentrations*, 2nd Edition (Beyer, W.N., and J.P. Meador, eds.), CRC Press, Boca Raton, Florida.

Table 1. Sentinel and Alternative Birds Captured and Sampled on the Savannah Dredge Material Containment Areas (DMCA) in the Summer (S) and Winter (W) Periods of Year 2 of Monitoring.

Sentinel Species	Sentinel Season	Spring-Summer	Fall-Winter
American Avocet[a]	W	-	6
Black-necked Stilt[a]	S	9	-
Brown Thrasher	Both	9	0
Eastern Towhee	Both	3	4
Mottled Duck[a]	S	4	-
Mourning Dove	S	9	-
Northern Mockingbird	Both	7	2
Northern Shoveler[a]	W	-	8
Red-winged Blackbird	S	9	1[b]
Savannah Sparrow	W	-	15
Song Sparrow	W	-	12
Alternative Species			
Common Ground Dove	S	2	-
Northern Cardinal	Both	1	6
Rock Pigeon	S	2	-
Swamp Sparrow	S	-	2

[a]These species are typically associated with wet conditions/aquatic habitats, although all species utilize edge habitat between aquatic and terrestrial habitats.

[b]Summer sentinel species captured in Winter.

Table 2. Avian Blood Concentrations (µg/g wet) of Cadmium from Birds Captured on the Savannah Harbor DMCAs Relative to the Method Detection Limits in 2015-2016 (Year 2 of Monitoring).

SPECIES	SEASON	N	# (%) < MDL	Max	MDL Range[a]
Sentinel					
American Avocet	Winter	6	5(83%)	0.01	0.001-0.018
Black-necked Stilt	Summer	9	6 (67%)	0.02	0.003-0.034
Brown Thrasher	Summer	9	1 (11%)	0.04	0.007-0.014
	Winter	0	-	-	-
Eastern Towhee	Summer	3	1 (33%)	0.01	0.005-0.010
	Winter	4	3 (75%)	0.02	0.006-0.051
Mottled Duck	Summer	4	4 (100%)	-	0.002-0.011
Mourning Dove	Summer	9	4 (44%)	0.09	0.005-0.011
Northern Mockingbird	Summer	7	3 (43%)	0.01	0.004-0.017
	Winter	2	1 (50%)	0.02	0.005-0.009
Northern Shoveler	Winter	8	8 (100%)	-	0.001-0.002
Red-winged Blackbird	Summer	9	6 (67%)	0.02	0.004-0.015
	Winter	1	1 (100%)	-	
Savannah Sparrow	Winter	15	15 (100%)	-	0.001-0.020
Song Sparrow	Winter	12	12 (100%)	-	0.005-0.021
Alternate[b]					
Common Ground Dove	Summer	2	2 (100%)	-	0.005-0.009
Northern Cardinal	Summer	1	0 (0%)	0.01	
	Winter	6	6 (100%)	-	0.007-0.014
Rock Pigeon	Summer	2	0 (0%)	0.01	0.001-0.002
Swamp Sparrow	Winter	2	2 (100%)	-	0.009-0.011

[a]Sample specific Method Detection Limits (MDLs) factoring in sample volume, etc.

[b]Alternate species were captured/sampled in case other sentinel species proved difficult to capture.

Table 3. Cadmium Concentrations (µg/g dry wt) in Tissue Samples and Gizzard Contents of Sentinel Species Lethally Collected on the Savannah Harbor DMCAs in 2015/2016 (Year 2 of Monitoring).

	Cd Concentrations (µg/g)		
	Kidney	Liver	Gut Content
Species (# Samples)	Mean±St Dev (Max)	Mean±St Dev (Max)	Mean±St Dev (Max)
American Avocet (7)	17.080±10.204 (26.751)	1.169±0.634 (1.952)	0.407±0.157 (0.584)
Mourning Dove (9)	15.991±22.917 (62.323)	4.687±5.014 (13.414)	0.440+0.250 (0.831)
Northern Shoveler (9)	8.651±8.879 (23.191)	1.259±0.703 (2.267)	0.156±0.065 (0.273)
Mottled Duck (4)	0.792±0.936 (2.140)	0.149±0.099 (0.274)	0.108±0.126 (0.292)
Savannah Sparrow (1)	3.624	1.197	0.937
Song Sparrow (1)	15.066	1.850	0.565

[a]Savannah and Song sparrows were capture mortalities, all others were collected via shotgun.

Table 4. Correlations[a] of Kidney and Liver Cd Concentrations in Selected Species of Avifauna Collected on the Savannah Harbor DMCAs in 2015/2016 (Year 2 of Monitoring).

Species	N	*r*	Prob > |*r*|
American Avocet	7	0.6786	0.0938
Mourning Dove	9	0.9000	0.0009[a]
Mottled Duck	4	0.8000	0.2000
Northern Shoveler	9	0.9500	<0.0001[a]

[a]Examined with Spearman Rank correlations. Significant differences at $P < 0.05$.

Table 5. Concentrations (µg/g Cd dry wt) of Cadmium Found in Potential Avian Prey Items Collected on the Savannah Harbor DMCAs in 2015/2016 (Year 2 of Monitoring).

Group/ Species (N)[a]	Cd Concentrations (µg/g) Mean±St Dev (Max)
Plant Material	
Chinese Tallow Leaves (*Triadica*) (1)	0.959
Soda Apple (*Solanum*) Leaves (1)	16.670
Duckweed (Lemnaceae.) (1)	2.697
Seeds/Fruit	
Blackberry (*Rubus* spp.) (3)	1.381±1.100 (2.483)
Chinese Tallow Berries (1)	1.408
Goat's Rue (*Tephrosia* spp) seed (3)	0.248±0.102 (0.365)
Grass (Poaceae) seed (5)	0.375±0.493 (1.161)
Insects	
Potato Beetle Larvae (Leptinotarsa) (1)	24.771
Aquatic Leaf Beetle (Chrysomelidae) (1)	0.148
Cranefly Larvae (Tipulidae) (1)	0.224
Firefly Beetles (Lampyridae) (2)	0.684±0.094 (0.751)
Aquatic Prey	
Mosquitofish (*Gambusia* sp.) (3)	0.059±0.021 (0.081)
Shrimp (Decopoda) (3)	0.658±0.129 (0.741)

[a]N=number of composite samples analyzed for Cd.

Table 6. Cumulative Sentinel and Alternative Species Sampled in Years 1 and 2 on the Savannah Dredge Material Containment Areas (DMCA) in the Summer (S) and Winter (W) Periods.

Sentinel Species	Season	N	# (%) < MDL	MDL Range[a]
American Avocet	W	6	5 (83%)	0.001-0.018
Black-necked Stilt	S	19	16 (84%)	0.003-0.034
Brown Thrasher	S	15	2 (13%)	0.001-0.014
	W	3	1 (33%)	0.007-0.026
Eastern Towhee	S	9	1 (11%)	0.001-0.010
	W	8	4 (50%)	0.006-0.072
Mottled Duck	S	4	4 (100%)	0.002-0.011
Mourning Dove	S	19	11 (58%)	0.005-0.011
Northern Mockingbird	S	19	13 (68%)	0.001-0.020
	W	5	1 (80%)	0.005-0.018
Northern Shoveler	W	16	16 (100%)	0.001-0.003
Red-winged Blackbird	S	20	17 (85%)	0.001-0.015
	W	1[b]	1 (100%)	0.004
Savannah Sparrow	S	9[b]	7 (78%)	0.001-0.013
	W	25	25 (100%)	0.001-0.021
Song Sparrow	W	23	23 (100%)	0.005-0.021
Alternative Species				
Common Ground Dove	S	6	6 (100%)	0.005-0.009
Northern Cardinal	S	9	8 (89%)	0.001-0.022
	W	17	17 (100%)	0.007-0.014
Rock Pigeon	S	2	0 (0%)	0.001-0.002
Swamp Sparrow	W	2	2 (100%)	0.009-0.011

[a]Sample-specific Method Detection Limits (MDLs) factoring in sample volume, etc.

[b]Individuals were captured in their "off-season", generally near the transition period between seasons.

Figure 1. Dredge material containment areas (DMCAs) associated with the Savannah Harbor Expansion Project.

APPENDIX 1. Metal Concentrations (ppm wet wt) in Avian Blood Samples Collected on the Savannah Harbor DMCAs in Year 2 (Summer 2015-Winter 2016).

Species	Season	N		Cr	Ni	Cu	Zn	As	Se
SENTINEL SPECIES									
American Avocet	Winter	6	Mean	0.24	0.16	0.29	3.83	<MDL	1.44
			SD	0.31	0.20	0.31	2.99	<MDL	1.23
Black-necked Stilt	Summer	9	Mean	0.23	0.11	0.50	6.24	0.02	0.99
			SD	0.16	0.13	0.17	2.31	0.01	0.69
Brown Thrasher	Summer	9	Mean	0.23	0.11	0.50	7.52	0.02	0.69
			SD	0.16	0.08	0.17	2.42	0.01	0.25
Eastern Towhee	Summer	3	Mean	0.26	0.16	0.36	6.32	0.02	0.52
			SD	0.17	0.19	0.07	1.71	0.00	0.15
	Winter	4	Mean	0.72	0.37	0.53	7.93	< MDL	0.65
			SD	0.87	0.46	0.20	2.64	-	0.30
Mourning Dove	Summer	9	Mean	0.18	0.13	0.49	7.66	< MDL	0.57
			SD	0.14	0.10	0.21	3.36	-	0.34
Northern Mockingbird	Summer	7	Mean	0.23	0.19	0.67	7.63	0.01	0.70
			SD	0.18	0.18	0.32	1.30	0.00	0.40
	Winter	1	Mean	0.23	0.16	0.59	7.14	< MDL	< MDL
			SD	-	-	-	-	-	-
Northern Shoveler	Winter	9	Mean	0.10	0.09	0.39	3.58	0.11	0.64
			SD	0.10	0.11	0.11	0.86	0.05	0.12

Species	Season	N		Cr	Ni	Cu	Zn	As	Se
SENTINEL SPECIES	(cont'd)								
Red-winged Blackbird	Summer	9	Mean	0.18	0.11	0.39	9.04	0.02	1.58
			SD	0.05	0.09	0.11	6.29	0.00	1.06
Savannah Sparrow	Winter	15	Mean	0.82	0.64	0.80	12.40	<MDL	1.40
			SD	0.81	1.65	0.72	8.89	-	1.21
Song Sparrow	Winter	11	Mean	0.54	0.37	0.54	7.85	< MDL	1.55
			SD	0.29	0.19	0.16	1.97	-	0.72
ALTERNATE SPECIES									
Rock Pigeon	Summer	2	Mean	0.02	0.01	0.22	4.36	<MDL	0.24
			SD	0.01	0.01	0.01	0.46	-	0.11
Northern Cardinal	Summer	1	Mean	0.26	0.19	0.51	6.93	0.01	<MDL
			SD	-	-	-	-	-	-
	Winter	6	Mean	0.25	0.26	0.41	6.20	<MDL	0.22
			SD	0.09	0.17	0.09	1.32	-	0.35
Method Detection Limits				Cr	Ni	Cu	Zn	As	Se
(range of MDLs in ppm)				0.029-0.236	0.017-0.341	0.213-0.716	0.093-0.413	0.018-0.097	0.341-0.977

APPENDIX 2. Metal Concentrations (ppm dry wt) in Tissues and Gut Contents from Lethally-collected Avian Species on Savannah Harbor DMCAs in Year 2 (Summer 2015-Winter 2016).

Species	N	Tissue		Cr	Ni	Cu	Zn	As	Se
Am. Avocet	7	Liver	Mean	0.04	0.09	25.37	110.56	3.17	9.03
			SD	0.03	0.02	12.81	12.33	1.08	0.56
		Kidney	Mean	0.04	0.07	20.67	112.54	2.03	10.04
			SD	0.03	0.04	6.22	14.54	1.08	0.94
		Gut Cont.	Mean	8.52	2.60	19.86	102.70	14.67	5.52
			SD	6.43	2.05	3.28	11.09	11.62	3.22
Mottled Duck	4	Liver	Mean	<MDL	0.07	120.65	117.83	0.19	4.57
			SD	-	0.02	205.91	19.94	0.17	1.87
		Kidney	Mean	<MDL	<MDL	14.81	77.93	0.34	5.22
			SD	-	-	4.65	4.59	0.50	1.10
		Gut Cont.	Mean	1.78	0.50	2.46	15.82	1.12	0.63
			SD	0.78	0.33	1.43	5.16	0.59	0.21
Mourn. Dove	9	Liver	Mean	<MDL	0.04	15.89	89.84	0.05	2.48
			SD	-	0.08	6.32	9.34	0.05	1.17
		Kidney	Mean	<MDL	0.12	12.22	94.79	0.18	4.02
			SD	-	0.08	2.42	33.26	0.07	1.79
		Gut Cont.	Mean	0.37	0.57	5.83	13.85	0.35	0.54
			SD	0.41	0.47	5.59	4.92	0.39	0.11
N. Shoveler	9	Liver	Mean	0.08	0.10	130.24	122.55	0.94	6.53
			SD	0.11	0.04	122.30	20.58	0.59	1.00
		Kidney	Mean	0.04	0.07	18.95	85.14	1.21	6.80
			SD	0.04	0.03	2.74	8.95	0.61	1.52
		Gut Cont.	Mean	4.09	0.96	5.08	25.22	3.73	1.01
			SD	3.64	0.35	2.61	14.96	2.01	0.42
			Method Detection Limits (in ppm)	0.02	0.02	0.02	0.12	0.03	0.38

APPENDIX 3. Metal Concentrations (ppm dry wt) in Potential Avian Prey Items Collected From the Savannah Harbor DMCAs in Year 2 (Summer 2015-Winter 2016).

Tissue	N		Cr	Ni	Cu	Zn	As	Se
Mosquitofish	2	Mean	0.70	0.27	4.09	149.61	0.58	1.07
		SD	0.21	0.07	0.45	2.44	0.09	0.46
Shrimp	3	Mean	0.80	0.70	36.14	67.85	6.82	2.15
		SD	0.43	0.19	4.06	1.40	0.57	0.12
Potato beetle adults	1	Mean	0.06	0.76	16.72	79.77	0.08	<MDL
		SD	-	-	-	-	-	-
Rubus berries	3	Mean	0.16	3.59	7.38	19.39	<MDL	<MDL
		SD	0.09	1.17	1.06	2.68	-	-
Tephrosia seeds	4	Mean	<MDL	3.07	7.21	39.20	<MDL	<MDL
		SD	-	1.64	1.41	2.96	-	-
Grass seeds	5	Mean	0.09	4.03	5.59	37.13	<MDL	<MDL
		SD	0.03	5.66	1.22	9.40	-	-
Chinese tallow fruit	1	Mean	<MDL	6.04	11.91	26.74	0.07	<MDL
		SD	-	-	-	-	-	-
Chinese Tallow leaves	1	Mean	0.10	9.64	5.61	5,63	0.28	<MDL
		SD	-	-	-	-	-	-
Duckweed	1	Mean	1.31	9.40	1.97	23.60	4.62	0.74
		SD	-	-	-	-	-	-
Soda apple leaves	1	Mean	0.15	1.36	8.83	70.99	0.04	<MDL
		SD	-	-	-	-	-	-
MDL			0.13	0.10	0.07	0.10	0.05	0.52