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Mercury Occurrence in Prothonotary Warblers (*Protonotaria citrea*) Inhabiting a National Priorities List Site and Reference Areas in Southern Alabama

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Abstract. Mercury occurrence in prothonotary warblers (*Protonotaria citrea*) was evaluated over two years in southern Alabama. Mercury was found in warbler nestlings and adults inhabiting National Priority List (NPL) sites in McIntosh, Alabama. Food items that were collected from nestlings also contained elevated mercury. When mercury concentrations in soil, food, and nestling were plotted at each nest box location, the distribution of mercury in the three matrices yielded information that direct bioaccumulation factors could not. There were site differences in mercury accumulation in nestlings inhabiting the NPL sites. Nestling mercury accumulation correlated with solid mercury concentrations near the nest box where the nestling was raised. Trophic transport of mercury was poorly defined by mercury in food; however, closer examination of prey items shows that food source influences accumulation. Mercury distributions in matrices provide useful information of uptake that can be integrated with risk assessment endpoints.

Regulatory and health agencies have placed high priorities on evaluating and reducing risk to humans and wildlife from exposure to toxic chemicals including mercury (ATSDR 1999; Rada *et al.* 1990; Sample and Suter II 1999). Mercury ranks third on the CERCLA list of substances posing risks to human health (ATSDR 1999). Mercury exposures are considered to be widespread (Driscoll *et al.* 1994; Hanisch 1998) and have prompted human health advisories in several regions of the US. The primary risk of mercury exposure to terrestrial vertebrates is through ingestion of contaminated food.

Risk assessments at waste sites have been mandated to include ecological as well as human risks. Exposure of threatened and endangered species to hazardous wastes is also closely scrutinized within the regulatory process (CFR 1999; CFR 2001). Many threatened and endangered birds migrate across the Gulf of Mexico and enter North America to breed (Gauthreaux 1996). The coastal plains of Alabama, Mississippi, and Georgia contain excellent wildlife habitat and sparse human population, which provide excellent breeding habitat for Neotropical migratory birds. However, the areas with sparse populations are getting smaller due to industrial and residential development.

The presence of contaminants from industry in crucial habitats has created concern for the health of a wide array of wildlife, including prothonotary warblers (*Protonotaria citrea*; CFR 1999; CFR 2001). The risks to wildlife inhabiting areas with mercury and DDT contamination in soils and sediments at a site in Alabama resulted in United States Fish and Wildlife Service (USFWS) studies considering potential natural resource damages to threatened and endangered species in this area. In a parallel ecotoxicology study, we examined mercury uptake and distribution in warblers at the same site.

Mercury uptake and effect in birds has been primarily measured for large migratory birds (Franson *et al.* 1999), Shorebirds (Braune *et al.* 2001; Kahle and Becker 1999; Muir *et al.* 1999; Gochfeld 1997), and raptors (Pain *et al.* 1999; DesGranges 1998; Hughes *et al.* 1997). Few studies have addressed these issues in passerines (Bishop *et al.* 1995; Rosten *et al.* 1998; Reynolds *et al.* 2001). The above studies agree that mercury accumulation occurs in all feeding guilds, and uptake is seen regardless of the matrix (blood, specific organ,feathers) examined. In arid environments, the only clear and positive correlations between mercury uptake and avian feeding guild position were shown in feather and egg mercury (Burger and Gochfeld 1996). Mercury form or availability control accumulation rates and could explain why mercury uptake and feeding guild correlations may not always work. Osprey nestlings from watersheds with recently constructed hydroelectric reservoirs accumulated higher mercury concentrations in tissues than did nestlings from older constructed reservoirs (Desgrange *et al.* 1998) or naturally formed reservoirs (Hughes *et al.* 1997). Contaminant studies with passerines have shown that resident passerine species at contaminated sites accumulate more mercury than do migratory or seasonal passerines (Rosten *et al.* 1998). However, accumulation patterns suggest that nestling passerines are the best indicator of local contaminant trends (Bishop *et al.* 1995).

Our research was designed to document and model the extent of mercury accumulation in nestling passerines from heterogeneous exposure concentrations. These descriptive techniques

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could be used with other endpoints to assess risk. The indicator species for this study was the nestling prothonotary warbler, a neotropical migrant with declining breeding habitats in North America. During the breeding season, adults feed nestlings invertebrates from a relatively small foraging area (Reynolds *et al.* 2001). Nestlings are immobile and have no previous exposure; therefore, exposure of nestlings would be from the site. Other qualities making warblers a good indicator species are that they nest in artificial nest boxes and tolerate human interaction, which allows easy monitoring and sample collection.

Kidney was the target organ used to assess mercury accumulation, because regardless of mercury form, initial accumulation is in the kidney (Zalups 1996). Even though the kidney accumulates the greatest amount of mercury initially, there were limitations. The organs were collected for endpoints other than mercury accumulation, as part of a larger study. Nestlings were small and fledged in less than two weeks, which resulted in small organs with short-term accumulation. These limitations required us to improve the mercury analysis technique (Adair and Cobb 1999).

The study took place at chlor-alkali and DDT facilities that released mercury and DDT, respectively, into sensitive ecosystems of a flood plain along the Tombigbee River near McIntosh, Alabama (31° 15′ N, 87° 58′ W; Figure 1). Although contaminants were released into relatively confined areas, they were transported across 200 ha of bottomland hardwood ecosystems by seasonal flooding (Reynolds *et al.* 2001). An extensive database was created after the site was placed on the National Priorities List (NPL) to characterize mercury and DDT in soil and sediment across the NPL sites (Woodward-Clyde 1995; Williams 1994). We used mercury concentrations in soils from the preliminary studies to examine mercury distribution and accumulation in warblers at the site.

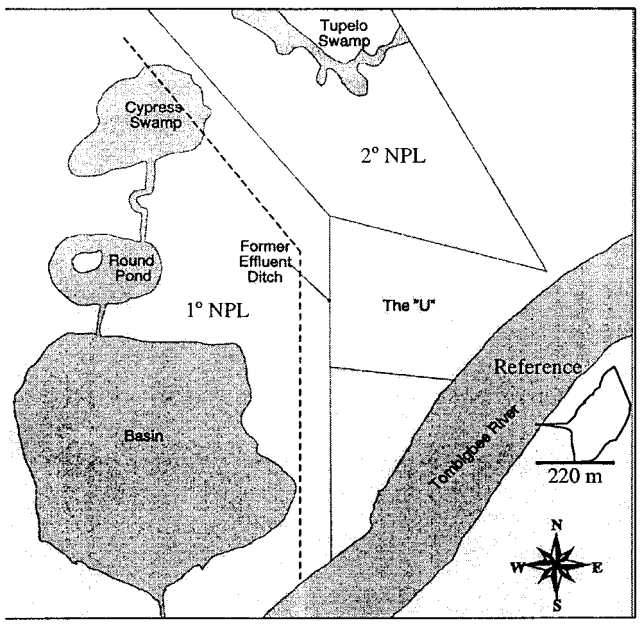

Fig. 1. Site map of NPL and Reference sites in Southern Alabama. The dashed line represents the property line between 1° NPL and 2° NPL. 2° NPL nest boxes were located along Tupelo Swamp, NE Cypress Swamp, and the ditches creating the "U." 1° NPL nest boxes were located around SW Cypress Swamp, Round Pond, and the Basin. Reference boxes were distributed around the primary water source and along the Tombigbee River

Materials and Methods

Field Methods

The study took place during the 1995 and 1996 breeding seasons. Samples were collected from adult warblers to evaluate mercury accumulation before and after inhabiting the study site. Before the 1996 breeding season, adult warblers migrating to the US were collected by mist netting at a coastal site in Bon Secour, Alabama (30° 14′ N, 87° 49′ W). At the conclusion of the 1996 breeding season, additional adult warblers were collected at their nests from Reference and NPL sites (ALD001221902 and ALD008188708).

One hundred nest boxes were placed 30 m apart along waterways at each site: 1° NPL, 2° NPL, and Reference sites (Figure 1). Nest boxes SW of the property line around Cypress Swamp were considered 1° NPL, and 2° NPL was located on the other side of the property line. The remaining boxes for 1° NPL were around Round Pond and the Basin, which contained the most mercury contamination in sediment and surrounding soil (Woodward-Clyde 1995, Williams 1994). Secondary NPL was composed of nest boxes around Tupelo Swamp and effluent/drainage ditches forming a U and connecting the U and Tupelo Swamp. Nest boxes were also placed along flooded regions at the reference site east of the U across the Tombigbee River. The position of each active nest box was located with a hand held global positioning system. The Universal Transverse Mercator (UTM) coordinates of boxes and soil sampling points on 1° and 2° NPL sites were used to calculate soil concentrations at active nest boxes and to create descriptive contour plots (Figures 2–4).

Nest boxes were monitored to determine nesting status, and to evaluate warbler-feeding habits. Food samples were collected from six and seven day old nestlings using constriction techniques that restricted food entry into the crop without limiting air intake (Cobb *et al.* 2000; Mellott and Woods 1993). Constrictors were placed around the nestling's neck above the crop and left in place for 30 min twice during the day. Food samples from each nest were pooled in acid rinsed glass vials and stored at −20°C until analysis. Invertebrate food items were identified at least to the order and often genus, before homogenization and residue analysis. Representative invertebrate food items from each year were removed from the pooled samples and analyzed separately for mercury content.

A randomly selected nestling was euthanized from each nest when nestlings were eight days old. Kidneys were removed from adult and nestling warblers within one hour of euthanization and stored under appropriate conditions for the analysis to be performed. One kidney from each warbler was placed in an acid cleaned glass vial (4 or 8 ml) with a Teflon coated lid. The other kidney was used for porphyrin analyses as part of a collaborative project.

Chemical Analysis

Mercury concentrations in food and kidney samples from nestling warblers were determined using methods developed for this project to maximize digestion efficiency of small samples at low concentrations and minimize sample loss and contamination. Detailed descriptions of validation, digestion, and analysis procedures are reported in Adair and Cobb (1999). Briefly, 0.1–0.3 g samples were predigested at room temperature in 1:1 HNO_3: H_2SO_4 in collection vials. Digests were

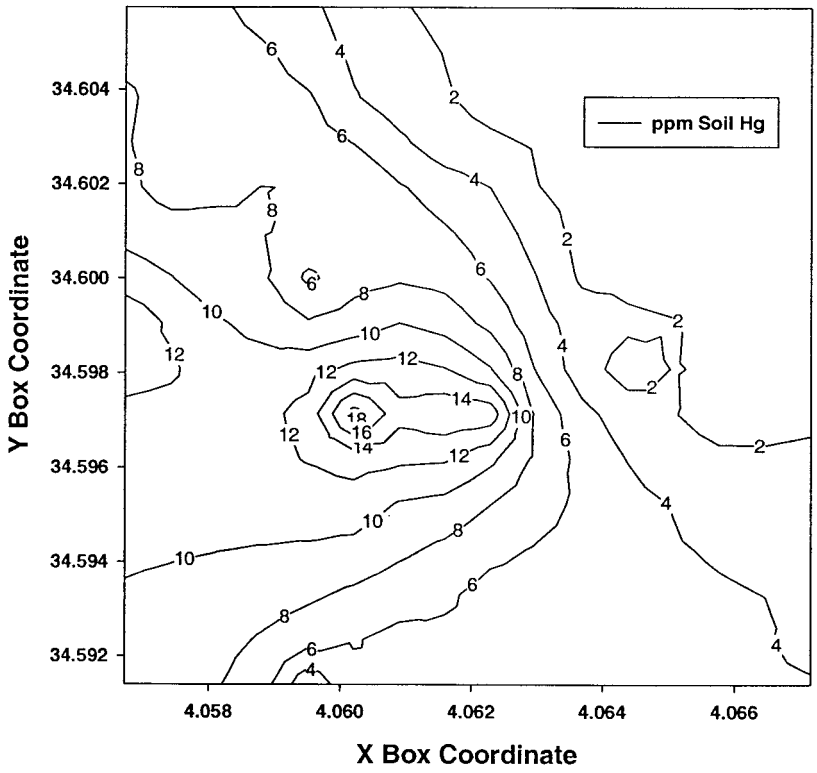

Fig. 2. Contour plot of soil mercury at active warbler nest box locations at NPL sites in Alabama. Coordinate system is UTM divided by 100,000. Soil Hg concentrations are geometric means of the four closest soil samples to the active box

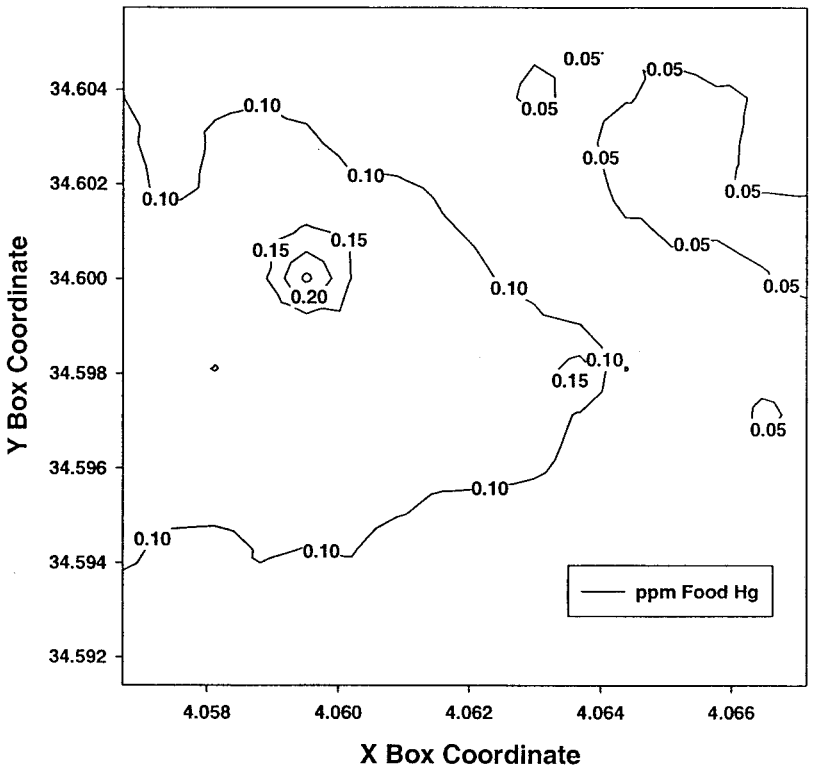

Fig. 3. Contour plot of food mercury at active warbler nest box locations at NPL sites in Alabama. Coordinate system is UTM divided by 100,000. The food mercury concentration was the pooled box concentration

heated in an 80°C water bath for one hour. Hydrogen peroxide (30%) was added to cooled digests that were reheated to 80°C. Digests were filtered and diluted to 10.00 ml with Milli-Q® water. Samples were analyzed using cold vapor atomic absorption (VGA 76, Varian Australia Pty. Ltd., Australia). Each analysis batch contained 40 warbler kidneys (or food samples), one spiked bovine kidney aliquot (for quality control), and one water blank. Duplicate analyses of warbler tissues were not possible given the small sample sizes and low con-

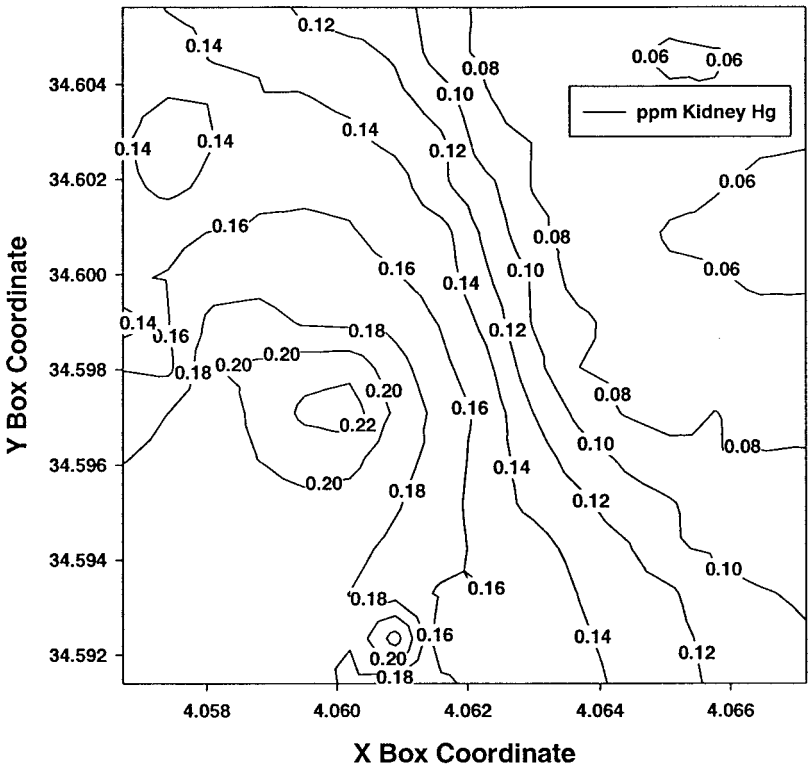

Fig. 4. Contour plot of kidney mercury at active warbler nest box locations at NPL sites in Alabama. Coordinate system is UTM divided by 100000. Kidney mercury was the box average of nestling kidney mercury concentrations

centrations of the tissues being analyzed. The average mercury recovery from the spiked bovine kidney homogenates was 99 ± 5% (n = 19), and the calculated method detection limit was 0.06 μg/g for a 0.15 g sample. Continuing calibration samples were also analyzed intermittently during each run. The instrument would have been recalibrated if the mercury concentration in the continuing calibration differed from its true concentration by >15%.

Data Analysis

The experimental unit was the nest box for this study; therefore, N values used to report kidney and food mercury data equal the number of boxes sampled unless stated otherwise. All mercury concentration data were log transformed before making statistical comparisons. Differences in mercury concentrations among sample groups were evaluated using one-way ANOVA procedures in SAS (SAS Institute Inc., Cary, NC). Site and year differences and interactions were examined using two-way ANOVA. Bonferonni post-hoc comparisons were performed if there were significant site differences to determine which sites were different.

As part of remedial investigations at the study sites, mercury concentrations in soil and sediment were available for 1,414 locations across the two NPL sites (Woodward-Clyde 1995; Williams 1994). Mercury concentrations in soil from the four sampling points nearest each nest box and the distances from each sampling point to the nest box were used to calculate a distance weighted mean soil mercury concentration at each nest box. The mean distance ± standard deviation of soil sampling points from nest boxes was greater at 2° NPL (220 ± 104 m, range 42–523 m) than the mean distance at 1° NPL (79.4 ± 33 m, range 15–78 m), because there were fewer soil samples collected for mercury analysis at 2° NPL. Soil samples were collected from the reference site; however coordinates were not available to create contour plots or perform partial correlations with residue data.

The uptake and distribution of mercury in soil, kidney, and food at the site were examined using partial correlation analysis. Partial correlation analysis examined the correlation between two matrices taking into account the influence of the third matrix (Sokal and Rohlf 1995). Partial correlation analysis was performed using Prostat (Poly Software Intl.; Salt Lake City, UT). To demonstrate the mercury distributions in the matrices, contour plots of mercury concentrations in soil, nestling food, and nestling kidney at each nest box were graphed using Sigma Plot 2000 (SPSS Inc.). Isopleths were created using meshed smoothing points. Smoothing points were weighted mercury concentration means from the inverse distance of neighboring points in the original data set. The sampling proportion of the mercury concentrations used to calculate the weighted means of each smoothing point was 0.5 (See Sigma Plot 2000, SPSS Inc. for more detailed description).

Results and Discussion

Adults were examined to determine the likelihood of mercury accumulation before inhabiting the study site. As adult warblers migrated into the US at the Gulf coast (Bon Secour), their kidneys contained 0.1282 mg/kg mercury (Table 1). Mercury accumulation in adults after inhabiting the study site was also examined. Adult kidneys from 1° NPL, 2° NPL, and Reference contained elevated mercury compared to the concentrations present following migration at Bon Secour ($p < 0.001$). Multiple comparisons revealed that mercury concentrations in adult warbler kidneys were different at each site (Table 1). These data demonstrate low mercury concentrations in adult warblers as they reach North America. The subsequent accumulation of mercury after inhabiting 1° NPL, 2° NPL, and Reference sites, was proportional to soil mercury.

Table 1. Geometric mean mercury concentration (ppm wet weight) in kidneys of adult warblers collected at the beginning (Bon Secour) and end (NPL and Reference sites) of the 1996 breeding season

Site	N[2]	Mean Kidney Hg[1] (95% CI[3])
Bon Secour	11	0.1282[a] (0.0748–0.1219)
1° NPL	3	1.5812[b] (1.2005–2.0825)
2° NPL	4	0.2835[c] (0.2266–0.3547)
Reference	6	0.1912[d] (0.1547–0.2364)

[1] Means with different letters were statistically different.
[2] N = number of individual adults collected from each site.
[3] CI = confidence intervals.

Table 2. Geometric mean mercury concentration (ppm wet weight) in kidneys of nestling prothonotary warblers in southern Alabama. Nestlings were collected from contaminated (NPL) and reference sites during the 1995 and 1996 breeding seasons

		Mean Kidney Hg[1] (95% CI[2])		Mean Kidney Hg (95% CI)
Site	N[3]	1995	N	1996
1° NPL	19	0.1688[a] (0.1354–0.2105)	12	0.1384[a] (0.1006–0.1904)
2° NPL	13	0.0476[b] (0.0363–0.0625)	12	0.0691[b] (0.0519–0.0920)
Reference	7	0.0259[c] (0.0121–0.0555)	18	0.0374[c] (0.0256–0.0546)

[1] There were no year differences and means with different letters were statistically different, $p < 0.05$.
[2] CI = confidence interval.
[3] N = number of nest boxes sampled.

Table 3. Geometric mean mercury concentration (ppm wet weight) in food items of nestling prothonotary warblers in southern Alabama. Samples were collected from contaminated (NPL) and reference sites during the 1995 and 1996 breeding seasons

		Mean Food Hg[1] (95% CI[2])		Mean Food Hg (95% CI)
Site	N[3]	1995	N	1996
1° NPL	18	0.0654[a] (0.0376–0.1138)	16	0.0468[a] (0.0312–0.0704)
2° NPL	12	0.0297[b] (0.0177–0.0498)	13	0.0288[b] (0.0177–0.0468)
Reference	8	0.0127[c] (0.0058–0.0280)	19	0.0262[c] (0.0195–0.0350)

[1] There were no year differences and means with different letters were statistically different, $p < 0.05$.
[2] CI = confidence interval.
[3] N = number of nest boxes sampled.

Adults had little if any previous exposure; therefore, nestlings were monitored for exposure and accumulation at the contaminated sites without concern of parental transfer. There were no year differences in nestling kidney mercury from all sites ($p = 0.0848$). During both years of investigation, nestlings from 1° NPL accumulated more mercury than nestlings from 2° NPL, which was greater than Reference sites ($p < 0.0001$; Table 2). Mercury accumulation in nestling kidneys at 1° NPL site was three to six times higher than accumulation at Reference site.

Food samples were examined to determine warbler exposure from ingestion. Mercury in food items demonstrated mercury ingestion by warbler nestlings that inhabited the NPL sites (Table 3). There were significant site differences of food mercury concentrations ($p < 0.001$). There were no year effects ($p = 0.3660$) or site by year interactions ($p = 0.2157$) of food mercury. Multiple comparisons of food mercury revealed significant differences among all sites (Reference < 2° NPL < 1° NPL).

Prey item identification was used to determine the primary food sources of the prothonotary warbler (Table 4). The major groups of invertebrates collected were terrestrial (diptera, lepidopteron larva), aquatic (ephimeraoptera), and arachnida. The aquatic species were more prevalent in 1995 than in 1996. Arachnids were present in more samples from 1996 than 1995. A representative set of spiders was analyzed separately for mercury content. When mercury contamination in spiders was compared to food samples with multiple invertebrate types, there were significantly higher mercury concentrations in spiders ($p < 0.001$; Table 5).

Mercury in invertebrate prey items confirms mercury exposure from food. To examine mercury accumulation from invertebrates to nestlings, bioaccumulation factors (BAFs) were calculated as direct ratios of food mercury to kidney mercury. BAFs should be independent of concentration and should therefore be consistent across sites. Individual box BAFs ranged from 0.20 to 23, and a two-way ANOVA for ranked data revealed no site difference ($p = 0.2261$) or year difference ($p = 0.5651$). The single food sample could not represent a cumulative dose, because of the variability of mercury concentrations in the different food types.

High variability of BAFs at individual nest boxes produced a poor description of mercury uptake; however, correlations using distributions could compensate for variability by examining the trend of mercury accumulation. Therefore, mercury distributions in soil (geometric mean), food items, and nestling kidneys were examined using partial correlation analysis (Table 6). Partial correlation analysis demonstrated the relationships between mercury concentrations within different matrices. Correlations of mercury in soil to kidney were significant ($p < 0.0001$), as were comparisons of mercury in soil to mercury food ($p < 0.001$). Mercury in soil described more of the mercury variation in kidney ($r = 0.6799$) than did mercury in food ($r = 0.3683$), which agreed with the spatial distributions. Mercury in kidney did not correlate significantly with mercury in food ($r = -0.0151$, $p = 0.9303$), which also agreed with spatial distribution data.

The influence that arachnids in food samples had on partial correlations was examined by performing separate statistical analyses for datasets with and without arachnids (Table 6). The overall probabilities of partial correlations separating datasets with and without spiders were the same as the total dataset. However, in datasets without arachnids, stronger correlations between mercury in soil and mercury in kidney ($r = 0.8482$,

Table 4. Invertebrate occurrence in food samples from nestling warblers in Southern Alabama. Samples were collected from two NPL sites and a Reference site over two years

	1995				1996			
Site	N[1]	Terrestrial[2]	Arachnid	Aquatic[3]	N	Terrestrial	Arachnid	Aquatic
1° NPL	18	100[4]	44.4	16.7	16	100	75	12.5
2° NPL	12	100	23.1	46.2	13	100	64.3	7.14
Reference	8	100	37.5	0	19	100	84.2	5.26

[1] N = total number of nest boxes sampled.
[2] Terrestrial invertebrates were predominately lepidopteron larvae.
[3] Aquatic invertebrates were composed primarily of odenota adults.
[4] Percent of food samples with specified invertebrate type.

Table 5. Geometric mean mercury concentration (ppm wet weight) in nestling prothonotary warbler food items separating spiders from other samples. Samples were collected from contaminated (NPL) and reference sites in southern Alabama during the 1995 and 1996 breeding seasons

		Mean Food Hg (95% CI[1])		Mean Food Hg (95% CI)
Site	N[2]	Spider	N	Homogenate[2]
1° NPL	4	0.0490 (0.0287–0.0837)	36	0.0424 (0.0294–0.0612)
2° NPL	6	0.0898 (0.0634–0.1272)	26	0.0230 (0.0159–0.0333)
Reference	7	0.2625 (0.1887–0.3651)	25	0.0184 (0.0126–0.0268)
All Sites[4]	17	0.1211[a] (0.1101–0.1333)	87	0.0278[b] (0.0271–0.0285)

[1] CI = upper and lower confidence intervals.
[2] N = number of individual food samples collected.
[3] Homogenate samples were pooled by box prior to analysis.
[4] Means with different letters were significantly different ($p < 0.05$).

Table 6. Results of partial correlation analysis of log Hg concentrations (ppm wet weight) in soil, kidney, and food samples collected from warbler nestlings in southern Alabama. Probabilities less than 0.05 are significant. Data from boxes with and without spiders present in food items were analyzed separately to examine effects of different food sources.

Dataset	N[1]	Matrices Correlated[2]	Coefficient ($r_{yx,z}$)	Probability
All data	36	Soil to kidney	0.68	<0.001
		Soil to food	0.368	0.002
		Kidney to food	−0.015	0.93
With Spider	23	Soil to kidney	0.537	<0.001
		Soil to food	0.427	0.003
		Kidney to food	0.049	0.821
Without Spider	13	Soil to kidney	0.848	<0.001
		Soil to food	0.426	0.041
		Kidney to food	−0.202	0.607

[1] N = number of nest boxes sampled.
[2] The analysis matrix not shown is held constant for partial correlation.

$p < 0.0001$) existed. Spiders represented a small fraction of the total food mass and were found sporadically, but spiders contained high mercury concentrations. Thus, food samples that contained spiders increased variance that reduced the ability of mercury in one matrix to explain mercury in another matrix. Future studies with extensive examination of diet composition and mercury in the separate components could increase the power of BAF and correlation analyses.

Contour plots were used to visualize mercury site distributions in the matrices. Coordinates for the contour plots were at nest boxes on 1° and 2° NPL sites (Figure 1). For descriptive purposes, contour plots of mercury concentrations in the three matrices at each active nest box on the two NPL sites were created (Figures 2–4). Distributions of mercury in soil and kidney samples appear similar (Figures 2, 4) and these similarities were confirmed by partial correlation analyses (see above). The location of the highest mercury concentration isopleths in both matrices occurs along the northwest bank of the basin (see Figure 1 for location description). The distribution of mercury in food items (Figure 3) was different from the distributions of mercury in kidneys and soil, as confirmed by partial correlation analyses (see above). The maximum mercury isopleths in food items occurred further north on the site, closer to cypress swamp.

Another perspective of contaminant uptake at the site used radio telemetry to determine adult warbler foraging areas for a more precise definition of exposure (Reynolds *et al.* 2001). Mercury uptake from soils within foraging areas was examined at several of nests, wherein all soil mercury concentrations from the preliminary studies were used to construct a more accurate mercury contour across the sites (Woodward-Clyde 1995; Williams 1994). Correlations of mercury in kidneys to concentrations in soil from specified foraging areas yielded slightly better results ($r = 0.88$, $p = 0.0003$; Reynolds *et al.* 2001) than the partial correlation analysis (Table 6) of mercury in soil and kidney. Although more extensive data was collected for foraging ranges and soil concentrations in the foraging area in Reynolds *et al.* (2001), fewer nests were examined and more equipment and labor were needed. The ease of data acquisition presented here provides a cost effective alternative for investigations that do not have the resources, time, or expertise to obtain foraging ranges of passerines.

Exposure and effect data from the literature were compared with our results to examine risk to the warblers inhabiting the site. The largest site mean for mercury in nestling warbler kidneys was 0.17 mg/kg at 1° NPL. Annual mean mercury concentrations of nestling warbler kidneys from 1° NPL were about one-tenth the 5 mg/kg dry weight (0.910 mg/kg wet weight) kidney mercury concentrations associated with nephrotoxicity in puffin (*Fratercula arctica;* Thompson 1996) and were over 100 times lower

than concentrations found to decrease avian reproductive success (US EPA 1997). The average mercury concentration in adult kidneys inhabiting the NPL sites from this study was 0.93 mg/kg, which was also significantly lower than concentrations found to cause adverse effects in avian species. Both adult and nestling kidney concentrations from Alabama were much less than 30 mg/kg kidney or liver mercury (wet weight) deemed harmful to bird of prey species (Thompson 1996).

Mercury accumulation in both adult and nestling warblers was demonstrated at the NPL sites, with greater accumulation in warblers inhabiting 1° NPL. Site distributions and partial correlations of mercury in soil, food items, and nestling kidneys revealed that mercury in food items and kidneys was best explained by mercury in soil. When compared to literature, mercury concentrations in both nestling and adult warblers suggest minimal risk of reproductive effects, organ toxicity, or survival to prothonotary warblers. This study demonstrates the utility of soil, kidney, and food mercury distributions with correlations to provide exposure assessment data for quantitative risk assessments.

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