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Hazard/Risk Assessment

The Spider Exposure Pathway and the Potential Risk to Arachnivorous Birds

Gale B. Beaubien,[a,*] Connor I. Olson,[b,c] Andrew C. Todd,[b] and Ryan R. Otter[a,b,d]

[a]Molecular Biosciences, Middle Tennessee State University, Murfreesboro, Tennessee, USA
[b]Department of Biology, Middle Tennessee State University, Murfreesboro, Tennessee, USA
[c]Department of Civil and Environmental Engineering, Syracuse University, Syracuse, New York, USA
[d]Data Science Institute, Middle Tennessee State University, Murfreesboro, Tennessee, USA

Abstract: There is growing concern over the health of North American birds, with evidence suggesting substantial population declines. Spiders are prominent dietary items for many bird species and mediate the transfer of contaminants to arachnivorous birds that consume them. Few studies have investigated the potential risk the spider exposure pathway poses to these birds because most studies have focused on piscivores. In the present study, we developed new chronic and acute As, Cd, Cu, Pb, Ni, Se, Zn, and MeHg spider-based avian wildlife values (SBAWVs) for multiple adult and nestling birds (primarily passerines) and then used the newly generated SBAWVs to characterize the risk to birds across 2 study areas: 1) 5 reaches in the southern Appalachian Mountains, an area with substantial mercury deposition but minimal anthropogenic impact, and 2) 4 reaches adjacent to the Emory River, an area impacted by the largest fly coal-ash spill in US history. We identified MeHg and Cu, Pb, Se, and Zn as contaminants of potential concern (COPC) at the Appalachian Mountain and Emory River study areas, respectively, based on dietary exposure of aquatic contaminants via riparian spiders. The identification of COPC at both study areas due to dietary spider exposure is notable not only because the spider exposure pathway has largely been uninvestigated at these sites but also because the aquatic systems in both areas have been studied extensively. Significant differences in MeHg concentrations were detected among spider taxa and suggest that the selection of spider taxa can impact risk characterization. These results indicate that the spider exposure pathway is important to consider when assessing potential risk, particularly for passerine birds. *Environ Toxicol Chem* 2020;39:2314–2324. © 2020 SETAC

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INTRODUCTION

In the United States, organism-level protection of nearly all bird species is mandated by the Migratory Bird Treaty Act of 1918 (2004), and substantive responsibilities have been imposed on federal agencies to "prevent or abate" environmental pollution for their benefit (Clinton 2001). Federal and state agencies operate under these statutes to determine the risk posed to birds by contaminants, and they initially assess the potential concern posed by uncharacterized sites and emerging contaminants using principles analogous to those of screening-level ecological risk assessments (US Environmental Protection Agency 1997, 1998a, 2001, 2003).

Because directly assessing ecological endpoints (e.g., reproduction) in birds can require considerable effort (Menzie et al. 1992), screening-level ecological risk assessments are used to evaluate the potential risks associated with contaminants and indicate whether a more detailed follow-up assessment is needed (US Environmental Protection Agency 2001). To characterize the potential risk to receptor organisms (in this case, birds), contaminant exposure is estimated for an identified exposure pathway (e.g., dietary), often by measuring the contaminant concentration in the exposure medium (e.g., dietary items). Then, contaminant exposure can be compared with an empirical toxic threshold, like a no-observed–adverse effect level or wildlife value through the calculation of a risk quotient (RQ): RQ = contaminant exposure/toxic threshold. When the calculated RQ is ≥1, the contaminant is considered a contaminant of potential concern (COPC), and a more detailed investigation with a higher level of effort is warranted (US Environmental Protection Agency 2001).

Spiders are prominent dietary items for many birds, and because they have the potential to mediate the transfer of

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* Corresponding author now with US Environmental Protection Agency, Office of Research and Development. Address correspondence to Beaubien.Gale@epa.gov
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bioaccumulative contaminants from their source (e.g., sediment or soil) to receptor organisms, like arachnivorous birds (Cristol et al. 2008; Walters et al. 2008; Jackson et al. 2011), the spider exposure pathway is considered a complete exposure pathway in screening-level ecological risk assessments (US Environmental Protection Agency 1997). Consideration for the spider exposure pathway may be particularly important for birds living and breeding near sites polluted with contaminants that biomagnify through aquatic food webs (e.g., methylmercury [MeHg] and polychlorinated biphenyls [PCBs]) because riparian spiders feeding on emergent aquatic insects feed at a relatively high trophic position compared with dietary items birds may otherwise consume (Cristol et al. 2008; Walters et al. 2010; Jackson et al. 2011; Speir et al. 2014; Gann et al. 2015). Additional consideration to nestlings is also warranted. In some instances, spiders may not be a core dietary item of a bird species' adult diet; however, even when this is this the case, several passerine species have still been known to preferentially provision spiders to their nestlings—often with peak provisioning occurring during early developmental stages (Royama 1970; Burger et al. 1999; Ramsay and Houston 2003; Arnold et al. 2007; Gunnarsson 2007; Radford 2008; Browning et al. 2012; García-Navas et al. 2013; Samplonius et al. 2016; Serrano-Davies and Sanz 2017).

Despite the large population declines seen in passerine birds across North America (Rosenberg et al. 2019), few studies have addressed the potential risk that the spider exposure pathway poses to arachnivorous birds. Walters et al. (2010) and Gann et al. (2015) examined the potential risk of this pathway using PCB and MeHg spider tissue concentrations, respectively; however, no studies have investigated the risk associated with this exposure pathway for a number of metals (i.e., Cd, Cu, Pb, Zn), despite laboratory and field evidence of their bioaccumulation in spiders (Otter et al. 2013; Yang et al. 2016). One explanation for this dearth of studies is that the development of spider-based avian wildlife values (SBAWVs) can be data-intensive to produce. These values are contaminant-specific and consider physiological and dietary differences between bird species, like life stage, body weight, ingestion rate, metabolic rate, and the percentage of spiders in the bird's diet. Thus, the calculation of SBAWVs is contingent on having the appropriate life-history data for the receptor species and a published toxic refence value (TRV) for the specified contaminant (US Environmental Protection Agency 1993, 1995). To date, SBAWVs have only been generated for 2 contaminants, PCBs and MeHg, and for only a few bird species and life stages (Walters et al. 2010; Gann et al. 2015).

Although it has become increasingly clear that riparian spiders are a tool that may be used to assess the potential risk of aquatic contamination to arachnivorous birds, uncertainty still exists over whether the spider taxa chosen for analysis are important. Past studies have relied on 2 riparian spider taxa to represent all spiders in risk calculations: araneids and tetragnathids (Walters et al. 2010; Gann et al. 2015). Only Walters et al. (2010) investigated both spider taxa in the calculation of bird risk, but their study focused solely on exposure to PCBs. To date, no study has examined the importance of different spider taxa in the calculation of MeHg risk to birds. Given the recent results of Ortega-Rodriguez et al. (2019) and Walters et al. (2018), which demonstrated that both MeHg and PCB concentrations can vary significantly among riparian spider taxa, it is important to determine whether taxa differences are great enough to warrant consideration in risk assessments.

In the present study, we calculated new chronic and acute As, Cd, Cu, Pb, Ni, Se, Zn, and MeHg SBAWVs for multiple adult and nestling birds and then used the newly generated SBAWVs to assess the potential risk to birds across 2 study areas. The first study area consisted of 5 reaches in the southern Appalachian Mountains, an area of high avian biodiversity and relatively high mercury deposition (wet and dry, see Selin 2009; Risch et al. 2017; Quintero and Jetz 2018). The second study area consisted of 4 reaches along the Emory River, where spider metal concentrations were measured following the largest coal-ash spill in US history (see Otter et al. 2013 for site description). The specific objectives of the present study were to 1) determine tetragnathid and araneid MeHg concentrations adjacent to 5 reaches within the Appalachian Mountain study area; 2) characterize the risk to arachnivorous birds across both study areas (all reaches) and determine which metals, if any, were COPC for arachnivorous birds; and 3) determine the influence of spider taxa on MeHg RQs and COPC determination for the Appalachian Mountain study area.

MATERIAL AND METHODS

Study areas

For the Appalachian Mountain study area, five 100-m study reaches were selected among 3 headwater streams that fall within the Unaka Range of Tennessee's Appalachian Mountains (Blue Ridge Mountains, ecoregion 66; Omernik 1995), an area known to have high levels of atmospheric mercury deposition (site map in Supplemental Data, Figure S1; Risch et al. 2017). Although as much as 98% of southern Appalachia has been altered directly by human disturbances (Gragson and Bolstad 2006), these 3 study streams are afforded some level of state or federal protection and have no documented upstream mining (US Geological Survey 2005). Four study reaches, Bald River-A, Bald River-B, Left Prong Hampton Creek-A (LPHC-A), and Left Prong Hampton Creek-B (LPHC-B), are located within the Cherokee National Forest. The fifth study reach, Rock Creek, is located within the Great Smoky Mountain National Park. In addition, each of these study reaches either directly overlaps or is in close proximity to the study reaches recently characterized as part of the Tennessee Wildlife Resources Agency's Tennessee's Ecologically At-Risk Streams project (Olson et al. 2019).

For the Emory River study area, all data were obtained from Otter et al. (2013). Four sampling locations were chosen, Emory

River mile 6 (ERM 6), Little Emory River mile 2 (LERM 2), Emory River mile 3 (ERM 3), and Emory River mile 1 (ERM 1; site map in Supplemental Data, Figure S2).

Spider collection and analysis (Appalachian Mountain study area)

Two riparian spider taxa were collected from the Appalachian Mountain study area: tetragnathids (*Tetragnatha elongata*) and araneids (*Araneus* spp.). Tetragnathids, also known as long-jawed orb-weavers, are land–water interface specialists that spin a horizontal web directly above the water's surface (Levi 1981; Gillespie 1987; Aiken and Coyle 2000) and feed primarily on adult aquatic insects (Gillespie 1987; Chaves-Ulloa et al. 2016; Kautza and Sullivan, 2016). Araneids are vertical web builders that have been shown to rely less on aquatic prey items than tetragnathids (Kato et al. 2003). All spiders were collected by hand in August of 2015. Collections took place at least 1 h after sunset from overhanging vegetation and structures within 1 m of the shoreline. At each stream reach, spiders were placed into taxon-specific polypropylene tubes, placed in a cooler with dry ice, and transported to the lab where they were sorted into taxon-specific composite samples ($n = 3$/reach) of multiple animals for Hg analysis. Laboratory processing consisted of confirming spider taxonomy (see Beaubien et al. 2019 for details on tetragnathid identification) and weighing each spider replicate to ensure that proper biomass was obtained for Hg analysis.

All tetragnathid and araneid composite samples with <2 g biomass ($n = 12$) were homogenized and analyzed for total Hg according to Method 1631 (US Environmental Protection Agency 2002). Briefly, composite samples were homogenized and stored frozen in acid-cleaned glass fluoropolymer jars. Samples were then transferred to a digestion vessel and then digested with HNO_3 and H_2SO_4 on a 58 °C hot block for 1 h. Once cooled, samples were diluted with 0.02 N BrCl and left at room temperature for an additional 4 h. Prior to analysis, an initial calibration verification was performed, and a continuing calibration verification was performed every 10 samples. Analysis of total Hg was conducted utilizing an Analytik Jena automated Hg analyzer. All samples were analyzed alongside method blanks (all reagents), a laboratory control sample, and a laboratory control sample duplicate. Method blanks were undetectable (<0.32 ng/g) and below the method reporting limit of 1.1 ng/g wet weight. Mean ± standard deviation (SD) percentage of recovery for laboratory control sample and laboratory control sample duplicate were 92 ± 8 and 99 ± 8%, respectively. The mean ± SD relative percentage of difference of the paired laboratory control sample and laboratory control sample duplicates was 7 ± 2%, and all were within acceptable ranges. Sample quality assurance included the use of the standard reference material (SRM) TORT-3, which was used to make one SRM blank, and matrix spike and matrix spike duplicates. In all cases, the percentage of recovery of the SRM blank, matrix spike, and matrix spike duplicate were within the acceptable range. Mean ± SD percentage of recovery of matrix spike and matrix spike duplicate were 99 ± 5 and 99 ± 5%, respectively.

Tetragnathid and araneid samples with >2 g biomass ($n = 18$) were analyzed for total Hg according to Method 7473 (US Environmental Protection Agency 1998b) utilizing a milestone direct mercury analyzer (DMA-80). These samples were analyzed alongside a method blank. The method blank was below the method detection limit of 0.032 mg/kg and below the method reporting limit of 0.020 mg/kg wet weight. Sample quality assurance included the use of TORT-3, which was used to make SRM blanks, matrix spike, and matrix spike duplicate. In all cases SRM blanks, matrix spike, and matrix spike duplicate were within the acceptable range. Mean ± SD percentage of recovery for matrix spike and matrix spike duplicate were 89 ± 4 and 91 ± 2%, respectively. Each paired matrix spike and matrix spike duplicate was within an acceptable range for relative percentage difference.

Spider collection, analysis, and dry weight conversions (Emory River study area)

The methodologies for collection and analysis of spiders from the Emory River study area are described in Otter et al. (2013). Briefly, tetragnathid spiders were collected by hand in July 2012, dried, and analyzed for a suite of total metal concentrations using inductively coupled plasma mass spectrometry (US Environmental Protection Agency 2007) and MeHg using cold vapor atomic fluorescence (US Environmental Protection Agency 1998c). Dry weight concentrations reported in Otter et al. (2013) were converted to wet weight using the mean reach-specific percentage of moisture (personal communication, R.R. Otter):

$$\text{Wet weight concentration} = \text{dry weight concentration} \\ /(100/100 - \%\ \text{moisture})$$

Reach-specific wet weight concentrations for As, Cd, Cu, Pb, Ni, Se, Zn, and MeHg as well as mean percentage of moisture are reported in Supplemental Data, Table S1.

Determination of receptor species (bird species)

A preliminary search for dietary data on birds that breed and/or presently (or recently) reside in the Appalachian Mountain study area was conducted by reviewing primary research and utilizing The Cornell Lab of Ornithology's Birds of North America online database to determine candidate birds to include in the present study. Candidate species were then vetted to ensure that all species-specific data were available to develop SBAWVs (i.e., mass, ingestion rate, and dietary information).

The SBAWVs were calculated for the following birds: adult and nestling American kestrels (*Falco sparverius*), adult brown creepers (*Certhia americana*), adult and nestling prairie warblers (*Setophaga discolor*), adult marsh wrens

TABLE 1: Species and life stage specific values used to calculate spider-based avian wildlife values

Order–family, common name (species)	Life stage	BW (g)	FMR (kJ/d)	IR (g/g/d)	S
Falconiformes–Falconidae					
American kestrel (*Falco sparverius*)	Adult	109[a]	256.3	0.427	4%[b]
	1–3 d	20[b]	80.8	0.733	5%$_{L}$,[b] 12%$_{H}$[n]
	7–10 d	55[b]	160.8	0.531	5%$_{L}$,[b] 12%$_{H}$[n]
Passeriformes–Certhiidae					
Brown creeper (*Certhia americana*)	Adult	8.55[c]	44.8	0.95	5.7%[o]
Passeriformes–Paridae					
Chickadee (*Poecile* spp.)	1 d	1[b]	10.4	1.888	25%[b]
	12 d	2[b]	16.7	1.513	25%[b]
Passeriformes–Parulidae					
Prairie warbler (*Setophaga discolor*)	Adult	8.78	45.6	0.942	6%$_{L}$,[d] 9%$_{H}$[d]
	1 d	2.11[d]	17.3	1.487	1%$_{L}$,[d] 9%$_{H}$[d]
	3–4 d	3.61[d]	24.9	1.252	1%$_{L}$,[d] 9%$_{H}$[d]
	12 d	6.46[d]	37.0	1.039	1%$_{L}$,[d] 9%$_{H}$[d]
Passeriformes–Passerellidae					
Field sparrow (*Spizella pusilla*)	1 d	2.28[e]	18.2	1.45	6%$_{L}$,[e] 12%$_{H}$[e]
	2 d	3.52[e]	24.5	1.262	6%$_{L}$,[e] 12%$_{H}$[e]
	3 d	5.05[e]	31.3	1.125	6%$_{L}$,[e] 12%$_{H}$[e]
	4 d	6.53[e]	37.3	1.036	6%$_{L}$,[e] 12%$_{H}$[e]
	5 d	7.87[e]	42.3	0.976	6%$_{L}$,[e] 12%$_{H}$[e]
Passeriformes–Troglodytidae					
Marsh wren (*Cisothorus palustris*)	Adult	10[f]	49.8	0.904	15.1%[f]
House wren (*Troglodytes aedon*)	Adult	10.6[g]	60.8[d]	1.041	26%[b]
	10 d	10[h]	49.8	0.904	9%[p]
Carolina wren (*Thryothorus ludovicianis*)	Adult	20[i]	79.8	0.724	31%[i]
Passeriformes–Turdidae					
Eastern bluebird (*Sialia sialis)*	Adult	30[i]	105.1	0.636	21%[b]
	2 d	3.9[j]	26.2	1.22	30.9%[q]
	5 d	10.75[j]	52.3	0.88	30.9%[q]
	8 d	18.45[j]	75.5	0.74	11.5%[q]
	14 d	26.8[j]	97.3	0.66	6.8%[q]
American robin (*Turdus migratorius*)	2 d	12.6[k]	58.3	0.839	2.3%[k]
	4 d	24.3[k]	91.0	0.68	2.3%[k]
	8 d	50.9[k]	150.5	0.537	2.3%[k]
	10 d	55.2[k]	159.1	0.523	2.3%[k]
	14 d	55.0[k]	158.7	0.524	2.3%[k]
Piciformes–Picidae					
Red-cockaded woodpecker (*Picoides borealis*)	Adult	48[l]	146.6	0.554	2%$_{L}$,[r] 8%$_{M}$,[r] 15%$_{H}$[r]
	9–12 d	33.8[m]	115.5	0.62	4.5%$_{L}$,[s] 11.4%$_{M}$,[s] 60%$_{H}$[s]

[a]Bloom (1973).
[b]Walters et al. (2010).
[c]Dunning (1984).
[d]Nolan (1978).
[e]Best (1977).
[f]US Environmental Protection Agency (1993).
[g]Dykstra and Karasov (1993).
[h]Fredricks et al. (2011a).
[i]Cristol et al. (2008).
[j]Roby et al. (1992).
[k]Howell (1942).
[l]Koenig et al. (2005).
[m]Stangel and Lenartz (1988).
[n]McDermot (2016).
[o]Otvos and Stark (1985).
[p]Fredricks et al. (2011b).
[q]Pinkowski (1978).
[r]Hess and James (1998).
[s]Hanula and Engstrom (2000).
Unless referenced, all FMRs were calculated using allometric equations provided by Nagy et al. (1999). For Falconiformes and Piciformes, FMR was calculated using the "all birds" equation: 10.5 × BW 0.681. For Passeriformes, FMRs were calculated using the "passerine" equation: 10.4 × BW 0.68.
Percentage of spider diets are shown as the average (or reported values) unless specifically indicated by an upper-case subscript for low (L), medium (M), and/or high (H).
BW = body weight; FMR = field metabolic rate; IR = ingestion rate; S = percentage of spider diet.

(*Cistothorus palustris*), adult Carolina wrens (*Thryothorus ludovicianus*), adult and nestling house wrens (*Troglodytes aedon*), adult and nestling eastern bluebirds (*Sialia sialis*), adult and nestling red-cockaded woodpeckers (*Picoides borealis*), nestling chickadees (*Poecile* spp.), nestling field sparrows (*Spizella pusilla*), and nestling American robins (*Turdus migratorius*); species-specific data are provided in Table 1.

SBAWV calculations

Chronic and acute MeHg SBAWVs were calculated considering contaminant exposure from spiders using the equation

$$\mathrm{SBAWV} = (\mathrm{TRV} \times [(\mathrm{IR}) \times \mathrm{S}]^{-1}) \times 1000$$

where SBAWV (nanograms per gram) is the contaminant concentration in spiders that is expected to cause a physiologically significant effect to birds; TRV is the toxic reference value (milligrams per kilogram per day), associated with an adverse response; IR is the ingestion rate, or the wet mass of prey ingested per day normalized to 1 g of body weight; and S is the percentage of spiders (by mass) normally found within the diet of the given species at a specific age or life stage (e.g., adult).

TRV

The SBAWVs were calculated using the TRVs recommended for birds by the Region 9 Biological Technical Assistance Group (US Environmental Protection Agency 2009). The Biological Technical Assistance Group-recommended chronic and acute values are provided in Supplemental Data, Table S2.

IR

The IR (grams per gram per day) was calculated using the formula

$$\mathrm{IR} = \{(\mathrm{FMR}/\mathrm{DM}) * [100/(100 - \%\text{-moisture})]\}/\mathrm{BW}$$

where FMR is the field metabolic rate (kilojoules per day), referenced from the literature or calculated using the referenced species- and age-specific body weights and allometric equations provided by Nagy et al. (1999), and DM is dry mass (kilojoules per gram) and represents the amount of metabolizable energy provided by each gram of prey. In each calculation, the suggested value of 18.0 kJ/g for insectivorous birds and reptiles was used (Nagy et al. 1999). To account for the difference of water in the food, 69.4% moisture in spiders (Gann et al. 2015) was used to calculate the amount of fresh matter ingested per day. Finally, BW is the species-specific and age-specific body weight (grams). The referenced species- and age-specific body weight used to calculate FMR is used to normalize ingestion to 1 g of body weight.

Percentage of spider diet (S)

Percentage of spider diet is the percentage of the bird's diet that is expected to be spiders (by mass). The values were calculated using the best available data for each respective life stage (see Table 1). When a range of percentage of spider diet was available, a range of SBAWVs were calculated for the appropriate life stages.

Data and risk analysis

For samples collected from the Appalachian Mountain study area, total Hg concentrations were converted to MeHg using a conversion of 70% of total Hg for tetragnathids (Otter et al. 2013; Tweedy et al. 2013) and 37% of total Hg for araneids (Wyman et al. 2011; Rodenhouse et al. 2019). Mercury residuals passed the assumption of normality, and a 2-way analysis of variance (ANOVA) was used to compare the main effects of spider taxa and reach and the interaction effect between spider taxa and reach on MeHg concentrations with a Tukey's post hoc analysis.

At each study reach, the potential risks of metals to arachnivorous birds were characterized using a deterministic approach. Reach-specific spider mean metal concentrations were used to estimate contaminant exposure to arachnivorous birds, and SBAWVs were used to estimate the toxic threshold. Risk quotients were calculated using the formula RQ = contaminant exposure/toxic threshold. Metals were established as a COPC at each respective reach when an RQ ≥ 1 was found for any of the respective bird species.

To determine whether the selection of taxa would influence RQ calculations and thus the establishment of MeHg as a COPC in the Appalachian Mountain study area, RQs were calculated using 1) the most conservative adult and nestling SBAWV for each respective receptor species and 2) spider taxon-specific MeHg concentration collected from each reach ($n = 3$/reach). Cube root transformations of adult and nestlings RQs were performed to meet the assumption of normality. Adult and nestling RQs were analyzed separately using a one-way blocked ANOVA, where spider taxon was considered a treatment, and receptor species were treated as blocks with fixed effects.

All statistical analyses were conducted using JMP 15.0 ($\alpha = 0.05$).

RESULTS AND DISCUSSION

There is growing concern over the health of North American birds, with evidence suggesting substantial population declines. Rosenberg et al. (2019) found that the total abundance of birds in North America has decreased by 29% since the 1970s, with passerines constituting the majority of these losses. These declines are likely due to multiple stressors (e.g., climate change, habitat loss), which may include metals and other environmental contaminants (Spiller and Dettmers 2019).

Across the Appalachian Mountain study area, mean tetragnathid and araneid MeHg concentrations ranged from 84.0 to 318.5 and from 13.9 to 58.8 ng/g wet weight, respectively. A significant reach–taxon interaction effect was observed ($F_{(4,29)} = 16.76$, $p < 0.001$), so main effects could not be meaningfully interpreted. Our post hoc analysis indicated that tetragnathid MeHg concentrations were significantly higher than araneid concentrations at every reach (Figure 1, connecting letters). Ortega-Rodriguez et al. (2019) similarly found differences in MeHg concentrations across several taxa of riparian spiders and attributed these differences to the degree of

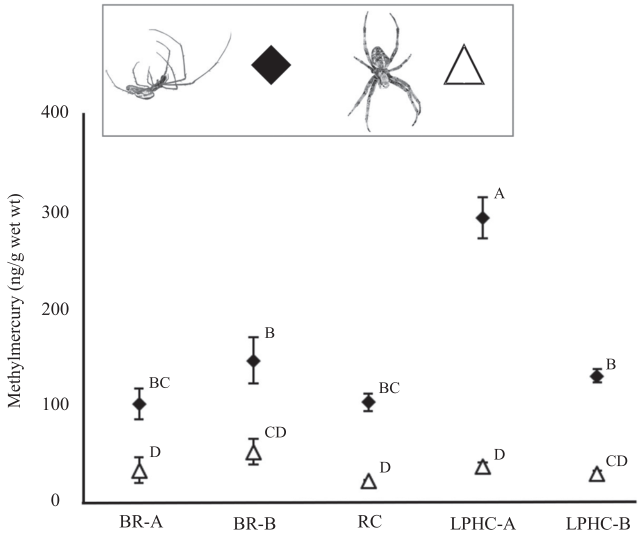

FIGURE 1: Methylmercury concentrations (mean ± standard error) in 2 spider taxa, tetragnathids (closed diamonds) and araneids (open triangles), collected from southern Appalachian Mountain reaches. Connecting letters indicate the differences among reach × taxa. BR-A = Bald River Reach A; BR-B = Bald River Reach B; LPHC-A = Left Prong Hampton Creek Reach A; LPHC-B = Left Prong Hampton Creek B; RC = Rock Creek.

dietary reliance on aquatic organisms. Although spider diet was not investigated in their study, differing feeding ecologies seems the most likely explanation for the concentration differences observed.

Chronic As, Cd, Cu, Pb, Ni, MeHg, Se, and Zn SBAWVs were calculated for adult and nestling birds (Table 2; acute SBAWVs are reported in Supplemental Data, Table S3). For every contaminant the most conservative adult and nestling SBAWVs were for the house wren and the 1-d chickadee, respectively. As a result, the designation of any metal as a COPC was contingent on exceeding the chronic 1-d chickadee SBAWV. These are the first published SBAWVs for all of these metals, with the exception of the MeHg SBAWV published by Gann et al. (2015). The chronic MeHg SBAWVs calculated in the present study were less conservative than those calculated by Gann et al. (2015), and these differences can primarily be attributed to the updated allometric equations (Nagy et al. 1999) and the use of different TRV values. Gann et al. (2015) used a more conservative TRV (0.013 mg/kg/d) derived from the Great Lakes water quality initiative and uncertainty factors proposed by Lazorchak et al. (2003), although the present study used a less conservative TRV recommended by the Biological Technical Assistance Group (0.039 mg/kg/d; US Environmental Protection Agency 2002). The use of overly conservative TRVs has garnered recent attention, and future studies and investigations should note that TRV selection unquestionably influences risk characterization (Fuchsman et al. 2017). The SBAWVs provided in the present study are intentionally conservative and are intended to screen potential risk—not provide a definitive assessment of actual or realized risk (US Environmental Protection Agency 2001).

A number of assumptions and factors need to be considered to properly contextualize the SBAWVs calculated in the present study. For example, the percentage of spiders in the diet and the type of spiders ingested are likely influenced by a suite of factors like spider availability, life stage, and other species-specific life-history traits that influence foraging strategy, which were not accounted for in SBAWV calculations (Gajdoš and Krištín 1997). In the present study, the data used for the percentage of a bird's overall diet that was composed of spiders were distilled to a single value for nestling chickadees, house wrens, and American robins; however, increased spider provisioning to younger–life stage nestlings has been reported with multiple bird species (Royama 1970; Pinkowski 1978; Cowie and Hinsley 1988; Arnold et al. 2007; Radford 2008; Browning et al. 2012), meaning that the SBAWVs calculated could be underprotective for younger nestlings (i.e., house wrens and red-cockaded woodpeckers). Many birds in the present study are also known to prey on adult aquatic insects (e.g., mayflies and dragonflies) and terrestrial insects (e.g., caterpillars and grasshoppers), which likely contribute additional, and unaccounted for, dietary contaminant exposure. The SBAWVs consider only the spider contaminant exposure pathways and assume that all nonspider dietary items are free of the contaminant (Walters et al. 2010; Gann et al. 2015; Williams et al. 2017).

The mean MeHg concentration in tetragnathids exceeded the most conservative chronic MeHg SBAWV (1-d nestling chickadees) at all reaches in the Appalachian Mountain study area; however, no exceedances were observed at any reaches in the Emory River study area. Therefore, MeHg was determined to be a COPC at all reaches in the Appalachian Mountain study area but at none of the reaches in the Emory River study area (Figure 2, top panel). With the exception of Bald River-A all reaches exceeded chronic MeHg SBAWVs for multiple receptor species. The reach with the most exceedances was LPHC-A, where exceedances also occurred for nestling chickadees (12 d), nestling prairie warblers (1 and 3–4 d), nestling field sparrows (1, 2, and 3 d), adult house wrens, nestling house wrens, adult Carolina wrens, adult eastern bluebirds, nestling eastern bluebirds (2 and 5 d), and nestling red-cockaded woodpeckers (RQs for all chronic MeHg SBAWVs are provided in Supplemental Data, Tables S4 and S5). Additionally, in all reaches but Bald River-A, spider concentrations exceeded both ages (1 and 12 d) for chickadee nestlings. This indicates that the potential risk to chickadee nestlings occurs over a large duration of the nestling life stage, including when they typically fledge (between 12 and 18 d; Cornell Lab of Ornithology 2019). No acute MeHg SBAWVs were exceeded at any reach in the Appalachian Mountain or Emory River study area (Supplemental Data, Tables S6 and S7; all RQs <1).

In the Emory River study area, As, Cd, Cu, Pb, Ni, Se, and Zn concentrations were assessed (Figure 2); and at all reaches Cu, Pb, Se, and Zn were established as COPC based on concentrations in tetragnathids exceeding the most conservative chronic SBAWV (1-d nestling chickadees; Figure 2). Chronic Cu and Se exceedances also occurred at every reach for adult house wrens. Additional exceedances were observed by other bird species for the metals deemed COPC. Briefly, exceedances occurred for nestling prairie warblers (Cu, Se), adult

TABLE 2: Chronic spider-based avian wildlife values (nanograms per gram) for adult and nestling birds

Order–family, common name (species)	Life stage	Diet type[a]	As	Cd	Cu	Pb	Ni	Se	Zn	MeHg
Falconiformes–Falconidae										
American kestrel (*Falco sparverius*)	Adult	Avg	322 141	41 000	134 714	820	80 828	13 471	1 007 424	2284
	7–10 d	Low	207 192	26 370	86 644	527	51 986	8664	647 946	1469
	1–3 d	Low	150 047	19 097	62 747	382	37 648	6275	469 236	1064
	7–10 d	High	86 330	10 987	36 102	220	21 661	3610	269 977	612
	1–3 d	High	62 519	7957	26 144	159	15 687	2614	195 515	443
Passeriformes–Certhiidae										
Brown creeper (*Certhia Americana*)	Adult	Avg	101 549	12 924	42 466	258	25 480	4247	317 573	720
Passeriformes–Paridae										
Chickadee (*Poecile* spp.)	12 d	Avg	14 545	1851	6082	37	3649	608	45 486	103
	1 d	Avg	11 652	1483	4872	30	2923	487	36 438	83
Passeriformes–Parulidae										
Prairie warbler (*Setophaga discolor*)	Adult	Low	97 295	12 383	40 687	248	24 412	4069	304 628	690
	12 d	Low	529 173	67 349	221 291	1347	132 774	22 129	1 654 868	3752
	3–4 d	Low	439 264	55 906	183 692	1118	110 215	18 369	1 373 698	3115
	1 d	Low	369 908	47 079	154 689	942	92 813	15 469	1 156 803	2623
	Adult	High	29 188	3715	12 206	74	7324	1221	91 280	207
	12 d	High	58 797	7 483	24 588	150	14 753	2459	183 874	417
	3–4 d	High	48 807	6212	20 410	124	12 246	2041	152 633	346
	1 d	High	41 101	5231	17 188	105	10 313	1719	128 534	291
Passeriformes–Passerellidae										
Field sparrow (*Spizella pusilla*)	5 d	Low	93 947	11 957	39 287	239	23 572	3929	293 799	666
	4 d	Low	88 500	11 264	37 009	225	22 206	3701	276 764	628
	3 d	Low	81 513	10 374	34 087	207	20 452	3409	254 912	578
	2 d	Low	72 622	9243	30 369	185	18 221	3037	227 107	515
	1 d	Low	63 199	8044	26 429	161	15 857	2643	197 641	448
	5 d	High	46 974	5978	19 644	120	11 786	1964	146 899	333
	4 d	High	44 250	5632	18 505	113	11 103	1850	138 382	314
	3 d	High	40 756	5187	17 044	104	10 226	1704	127 456	289
	2 d	High	36 311	4621	15 185	92	9111	1518	113 554	257
	1 d	High	31 600	4022	13 214	80	7929	1321	98 820	224
Passeriformes–Troglodytidae										
Marsh wren (*Cisothorus palustris*)	Adult	Avg	40 304	2585	16 854	103	10 113	1685	126 041	286
House wren (*Troglodytes aedon*)	Adult	Avg	20 314	8606	8495	52	5097	849	63 526	479
	10 d	Avg	67 621	8606	28 278	172	16 967	2828	211 469	479
Carolina wren (*Thryothorus ludovicianus*)	Adult	Avg	24 507	3119	10 248	62	6149	1025	76 640	174
Passeriformes–Turdidae										
Eastern bluebird (*Sialia sialis*)	Adult	Avg	14 572	5242	17 225	105	10 335	1722	128 810	292
	14 d	Avg	122 692	15 615	51 308	312	30 785	5131	383 692	870
	8 d	Avg	64 379	8194	26 922	164	16 153	2692	201 331	457
	5 d	Avg	20 157	2565	8429	51	5057	843	63 035	143
	2 d	Avg	14 572	1855	6094	37	3656	609	45 569	103
American robin (*Turdus migratorius*)	14 d	Avg	456 574	58 109	190 931	1162	114 559	19 093	1 427 833	3238
	10 d	Avg	457 105	58 177	191 153	1164	114 692	19 115	1 429 492	3241
	8 d	Avg	445 395	56 687	186 256	1134	111 754	18 626	1 392 871	3158
	4 d	Avg	351 552	44 743	147 013	895	88 208	14 701	1 099 399	2493
	2 d	Avg	284 914	36 262	119 146	725	71 488	11 915	891 004	2020
Piciformes–Picidae										
Red-cockaded woodpecker (*Picoides borealis*)	Adult	Low	495 968	63 123	207 405	1262	124 443	20 740	1 551 026	3517
	Adult	Med	123 992	15 781	51 851	316	31 111	5185	387 756	879
	Adult	High	66 129	8416	27 654	168	16 592	2765	206 803	469
	9–12 d	Low	197 097	25 085	82 422	502	49 453	8242	616 375	1398
	9–12 d	Med	77 801	9902	32 535	198	19 521	3254	243 306	552
	9–12 d	High	14 782	1881	6182	38	3709	618	46 228	105

[a]Diet type column indicates the percentage of spider diet (S) referenced in Table 1: low, medium (Med), high, or average (Avg).
Toxic reference values were calculated using chronic spider-based avian wildlife values recommended by the US Environmental Protection Agency's Region 9 Biological Technical Assistance Group (US Environmental Protection Agency 2009). As = 5500 ng/g/d; Cd = 700 ng/g/d; Cu = 2300 ng/g/d; Pb = 14 ng/g/d; Ni = 1380 ng/g/d; Se = 230 ng/g/d; Zn = 17 200 ng/g/d; MeHg = 39 ng/g/d.

prairie warblers (Se), nestling field sparrows (Cu, Se), nestling eastern bluebirds (Cu, Pb, Se), adult eastern bluebirds (Se, Zn), nestling red-cockaded woodpeckers (Cu, Pb, Se, Zn), and adult marsh wrens (Se; all chronic RQs reported in Supplemental Data, Tables S8–S14). No acute SBAWVs were exceeded for any metal at any reach in the Emory study area (all RQs <1; Supplemental Data, Tables S15–S21). The chronic exceedances observed in the present study indicate that the spider contaminant exposure pathway may not be limited to contaminants that biomagnify but may also extend to metals expected to inhibit emergence. The results of the present study reinforce the recent work of Naslund et al. (2020), who found

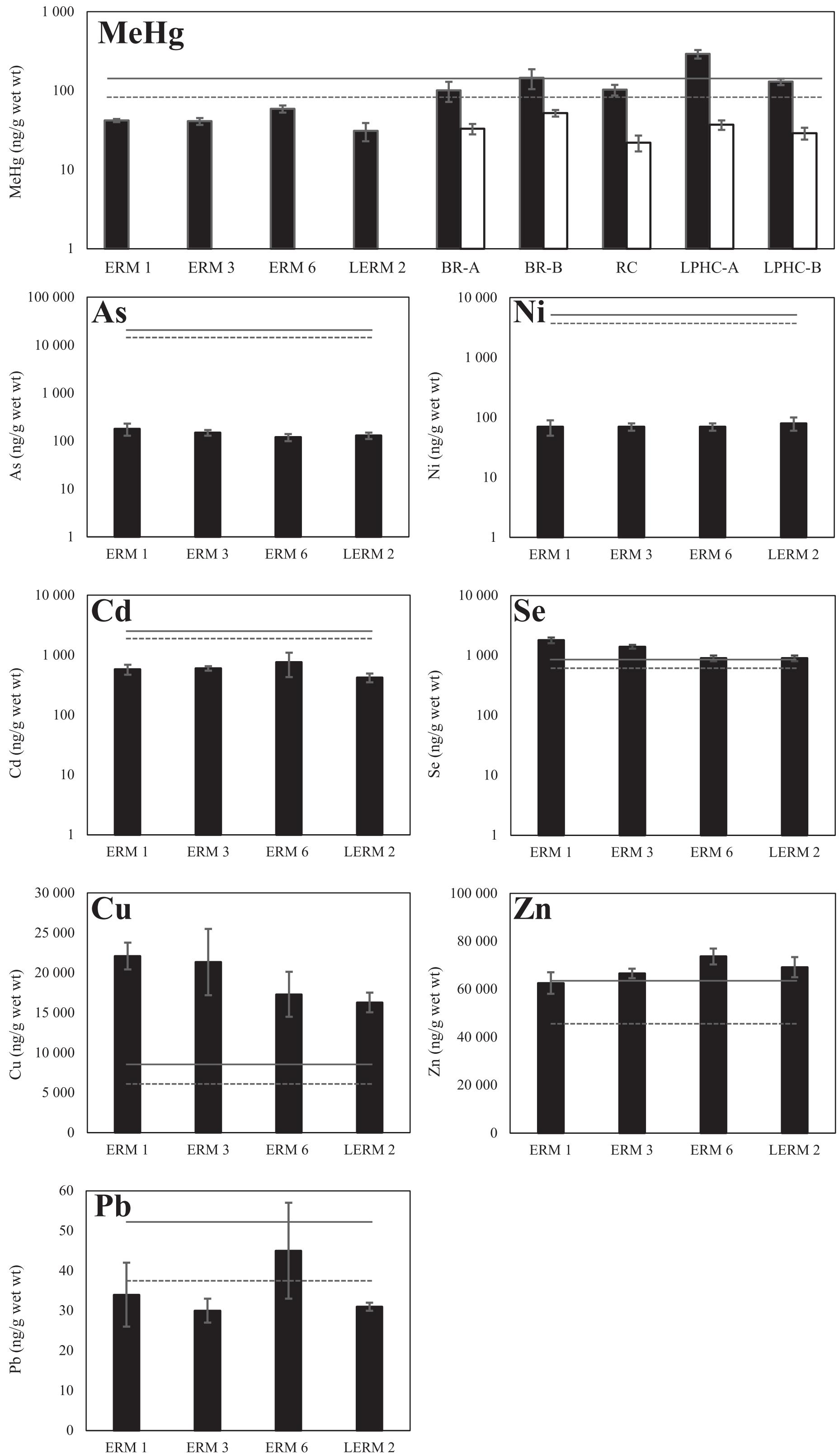

FIGURE 2: Riparian spiders, tetragnathid (black bars) and araneid (white bars), concentrations ± standard deviation for methylmercury, arsenic, cadmium, copper, lead, nickel, selenium, and zinc. Solid and dashed lines represent the most conservative spider-based avian wildlife value calculated for passerine adults (house wrens) and nestlings (chickadees), respectively. BR-A = Bald River Reach A; BR-B = Bald River Reach B; ERM 1 = Emory River Mile 1; ERM 3 = Emory River Mile 3; ERM 6 = Emory River Mile 6; LERM 2 = Little Emory River Mile 2; LPHC-A = Left Prong Hampton Creek Reach A; LPHC-B = Left Prong Hampton Creek B; RC = Rock Creek.

that even though aquatic insect emergence was negatively impacted by the high Se water concentrations and Se export via emergent insects was presumably low (sensu Schmidt et al. 2013), Se concentrations in tetragnathids could still exceed Se screening values for birds.

The identification of COPC at both study areas due to dietary spider exposure is notable not only because the spider exposure pathway has largely been uninvestigated at these sites but also because the aquatic systems in both areas have been studied extensively. Olson et al. (2019) investigated MeHg concentrations at 3 of the reaches used in the present study (Bald River-A, Rock Creek, LPHC-A) and found that all fish concentrations were below screening values for human consumption (0.3 mg/kg MeHg). Similarly, there have been numerous investigations into the effects of coal ash on the aquatic biota of the Emory River (Otter et al. 2012, fish; Otter et al. 2015, mussels); however, it remains unclear if there have been any substantial, long-term effects on fish health (Pracheil et al. 2016). The results of the present study indicate that if future studies focus only on aquatic organisms, they may overlook or improperly characterize risk if other receptor organisms like arachnivorous passerine birds are not considered. For future investigations into the potential risk of aquatic contaminant exposure to birds, we recommend that the spider contaminant exposure pathway be considered for use as a screening tool.

We assessed the impact of the species-specific differences in MeHg tissue concentrations on risk characterization and found a significant effect of spider taxa selection on risk characterization for adult and nestling birds (adults: $F_{(1, 231)} = 161.62$, $p < 0.001$; nestlings: $F_{(1, 231)} = 129.99$, $p < 0.001$). These differences had a practical effect on risk characterization—when RQs were calculated based on araneid tissue concentrations, MeHg was never designated as a COPC (all araneid chronic RQs <1; reported in Supplemental Data, Table S22). Conversely, when RQs were calculated based on tetragnathid tissue concentrations, MeHg was designated as a COPC at all reaches. These findings underscore the importance of considering taxon when designing and evaluating screening-level ecological risk assessments because the taxa sampled can have substantial effects on the conclusions reached. For sites contaminated with MeHg, we suggest that tetragnathid tissue concentrations are preferable to other taxa because they not only provide a conservative estimate of risk but also may provide an additional line of evidence that, if necessary, could be used to characterize the effectiveness of future remediation efforts (e.g., Kraus et al. 2017; Walters et al. 2018). We do note that the utility of tetragnathids is contingent on their abundance, and their use may be complicated by biomass requirements necessary to achieve target method detection limits (Beaubien et al. 2019). When tetragnathid-specific composite samples cannot alone provide the biomass necessary to achieve target method detection limits, the integration of other larger spider taxa, like araneids, may be unavoidable. If future studies characterize risk using tissue concentrations of alternative taxa, like araneids, or integrate multiple taxa into a composite sample, we recommend that the composition of the sample be considered when characterizing the potential risk posed via the spider exposure pathway.

CONCLUSION

The present study has presented an approach for assessing the potential risk posed to arachnivorous birds via the spider exposure pathway and the impact of spider selection on risk characterization. The methodology used for these calculations is flexible and can be applied to any bird species in any area for any contaminant so long as the requisite life-history and toxicological data are available. Notably, the present study demonstrated the value of considering the spider exposure pathway; when considering only the consumption of spiders at 2 very different study areas, the potential risk of adverse effects existed for a variety of metals (MeHg, Cu, Pb, Se, Zn). With populations of passerine birds on the decline, it is critical to account for previously understudied exposure pathways.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at https://doi.org/10.1002/etc.4848.

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