Arch Environ Contam Toxicol
DOI 10.1007/s00244-013-9913-5

Spatial and Temporal Variation in the Diet of Tree Swallows: Implications for Trace-Element Exposure After Habitat Remediation

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Received: 22 February 2013 / Accepted: 30 April 2013

Abstract Emerging aquatic insects play a key role in transporting aquatic contaminants into terrestrial ecosystems. Tree swallows are frequently the focus of studies examining this movement because they are thought to forage heavily on emerging aquatic insects when breeding in riparian areas. We examined the tree swallow diet to determine if trace elements from a recently remediated coal fly ash spill were moving into the terrestrial ecosystem. We collected bolus samples from adult tree swallows as they entered the nest box to feed their young. Despite strategically locating boxes in riparian areas, we found that the consumption of insects with an aquatic larval stage ranged from 28 to 75 % of insects among colonies. We also found significant differences among colonies in the taxa found in bolus samples. Chironomidae (midges) were the primary emerging aquatic insects consumed by tree swallows, whereas Ephemeroptera were brought to nestlings infrequently. The consumption of insects with an aquatic larval stage, Chironomidae in particular, was positively correlated with exposure to trace elements from the spill. Bolus samples from the spill site contained greater concentrations of many trace elements compared with reference locations, but concentrations of most elements were lower than levels thought to cause reproductive impairment. These results support the hypothesis that emerging aquatic insects transport trace elements to terrestrial consumers and that Chironomidae play an important role in this movement. Our results also indicate that it is important to assess the composition of the diet and to not infer exposure to trace elements based on nesting location.

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The movement of environmental contaminants through ecosystems and their potential effects on wildlife populations is of great conservation concern. Aquatic ecosystems often become concentrated sources of contaminants due to run-off from terrestrial habitats or industrial disposal practices that use water to dilute or hold contaminated materials (Rowe et al. 2002; United States Environmental Protection Agency 2002). Thus, much contaminant research focuses on aquatic or piscivorous species and the movement of contaminants through primarily aquatic ecosystems (Hasler 1975; Di Giulio and Tillet 1999; Albers et al. 2000; Krummel et al. 2003). However, freshwater streams produce an incredible biomass of emerging aquatic insects, which can average between 10,000 and 20,000 individuals/m^2/year (Jackson and Fisher 1986), and lakes typically produce even greater biomass (Gratton and Vander Zanden 2009). As a result, emerging aquatic insects provide important nutrient and energy subsidies to terrestrial consumers, including birds, bats, spiders, and amphibians (Sabo and Power 2002; Baxter et al. 2005; Fukui et al. 2006). Emerging aquatic insects also export contaminants from aquatic systems and introduce contaminants into terrestrial food webs (Vander Zanden and Sanzone 2004; Baxter et al. 2005).

Terrestrial animals that eat emerging aquatic insects can be at considerable risk of contaminant exposure and resulting toxicity (Menzie 1980; Runck 2007; Cristol et al. 2008; Walters et al. 2008). Terrestrial predators that forage heavily on aquatic insects in areas contaminated by biomagnifying pollutants, such as polychlorinated biphenyls (PCBs) and

Published online: 22 May 2013

mercury (Hg) (Kidd et al. 1995; Evers et al. 2005), show significant bioaccumulation of these contaminants that in some cases exceed the concentrations detected in higher trophic level consumers, such as piscivorous species, feeding in the same areas (Cristol et al. 2008). Additional research is needed to address the movement of other contaminants, such as a variety of trace elements, to determine if similar exposure risks exist for lower level trophic consumers as have been found for PCBs and Hg.

Pollution of aquatic systems may also alter the quality or quantity of invertebrate prey available to consumers. For instance, some species of Ephemeroptera (mayflies) are sensitive to increased trace element concentrations and exposure can shift the community to more metal-tolerant species, including species of Chironomidae (Diptera) (Winner et al. 1980; Clements et al. 1992; Clements et al. 2000). Trace elements can also decrease the quantity of invertebrate prey in contaminated areas and thus lead to decreases in growth and survival of amphibian and fish larvae (Cherry et al. 1979; Hopkins et al. 2004; Roe et al. 2006). In areas with metal contamination, preferred prey types may be in lower abundance or of lower quality, causing predators to switch to less preferred prey types (Eeva et al. 2005). Naturally occurring temporal or spatial variation in emerging aquatic insect hatches or in nutritional requirements during breeding can also cause dietary shifts (Dunn and Hannon 1992; Smits et al. 2005; Harding 2008; Morrissey et al. 2010). Dietary shifts during key periods of reproduction or development could alter the exposure of adults or young to environmental contaminants. Therefore, it is important to quantify contaminant exposure and the composition of the diet to determine how these factors affect the health of vertebrate consumers.

In December 2008, a coal fly ash impoundment at the Tennessee Valley Authority (TVA) fossil plant in Kingston, TN, ruptured releasing 4.13 million m^3 of coal fly ash into the Emory River, which then flowed into the Clinch and Tennessee Rivers (TVA 2009a). Coal fly ash contains increased concentrations of trace elements, such as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), selenium (Se), and vanadium (V), but the elements present and concentrations vary depending on the original composition of the coal and the combustion technologies used (Dvorak 1977, 1978; National Research Council 2006). Extensive dredging of the Emory River removed most of the fly ash from the system by August 2010; however, approximately 400,000 m^3 of ash remains in the system or was left in place to avoid disturbing legacy contamination, including cesium-137, PCBs, and Hg in the river sediments (TVA 2009b, 2011a). We examined the extent to which trace elements from this remediated fly ash spill can move into terrestrial consumers through the consumption of emerging aquatic insects using tree swallows (*Tachycineta bicolor*) as a model system.

Tree swallows are one of the primary species used to address the movement of contaminants, including trace elements, from aquatic to terrestrial ecosystems (Custer 2011). Tree swallows are secondary cavity nesters and readily settle in nest boxes (Robertson et al. 2011). Both sexes remain close to their nest site throughout the breeding season and typically forage within 300–500 m of their nest box (Quinney and Ankney 1985; Dunn and Hannon 1992). They are aerial insectivores, and emerging aquatic insects are thought to be a primary food source when breeding in riparian areas. They lend themselves to dietary studies because adults feeding nestlings retain food in their bill which can be easily removed on capture (Brasso and Cristol 2008). Insects in a bolus are often still alive and retain diagnostic features that permit identification to order and family.

The objective of this study was to determine if tree swallows breeding near the spill were exposed to increased concentrations of trace elements and to determine which arthropod taxa were primarily responsible for moving contaminants into terrestrial food webs. We collected bolus samples from several tree swallow colonies in the vicinity of the fly ash spill to identify the types and quantities of insects present. We predicted that the tree swallow diet would consist primarily of insects with an aquatic stage in their life cycle because our colonies were located exclusively along shorelines. We examined the effect of nestling age on the composition of the tree swallow diet and predicted that diet would change little with nestling age as found in other studies on tree swallows (McCarty and Winkler 1999; Bortolotti et al. 2011). We collected samples throughout the breeding season (May through July) to account for the fact that insect availability may change seasonally and thus affect the composition of the diet. Because mayflies typically emerge during warmer months and emergence is delayed by cold weather (Brittain 1982; Takemon 1990), we predicted that more mayflies would be consumed later in the season. We then tested the hypothesis that composition of the diet affected the exposure of tree swallows to trace elements in the system. We predicted that tree swallows in colonies exposed to ash that consumed more insects with an aquatic stage in their life cycle, particularly Ephemeroptera (mayflies) and Chironomidae (midges), would be exposed to greater concentrations of trace elements than birds that consumed primarily terrestrial insects.

Materials and Methods

Study Area and Bolus Collection

We established nest box–breeding colonies of tree swallows in Roane and Loudoun counties, TN. These colonies

encompassed a trace element contamination gradient that ranged from background exposure at reference colonies to potentially high exposure near the ash spill (Fig. 1). Colonies were defined as being impacted by the fly ash spill if they were located at or downstream from the spill, with colonies close to the spill considered high-impacted colonies and those farther downstream considered moderate- or low-impacted colonies. We placed nest boxes at two high-impacted colonies, the site of the spill (spill site, $N = 94$) and downstream from the spill at the confluence of the Clinch and Emory Rivers where ash was not dredged during remediation (hereafter downstream 1, $n = 31$). Two moderately impacted colonies were monitored farther downstream on the Clinch River (downstream 2) near the Kingston Fossil Plant discharge ($N = 31$) and Kingston City Park (downstream 3; $N = 43$) and were located approximately 3.0 and 7.0 km from the confluence with the Emory River, respectively. Downstream 4 was a low-impacted colony located approximately 2.5 km downstream from the confluence of the Clinch and Tennessee Rivers ($N = 51$). Reference colonies were located at Ft. Loudoun Dam on the Tennessee River approximately 30.5 km east of Kingston (reference 1, $N = 46$) and at Tellico Dam (reference 2, $N = 53$) on the Little Tennessee River. The third reference colony was located at Long Island (reference 3, $N = 53$) approximately 5.5 km upstream on the Tennessee River from the confluence with the Clinch River. We also placed boxes at Melton Hill Dam ($N = 68$), 30.5 km northeast of Kingston, TN on the Clinch River. This colony served a role analogous to a positive control because preliminary data gathered before this study indicated that tree swallow young and eggs had increased concentrations of trace elements associated with fly ash, such as Se (ARCADIS 2011). The probable source(s) of this contamination were unclear but included a former coal ash storage pond associated with the Y-12 Security Complex (Cook et al. 1999), the Bull Run Fossil Plant (Stantec 2009; TVA 2011b), or other non–point source pollution (United States Department of Agriculture 2009) (USDA) and warrant further investigation.

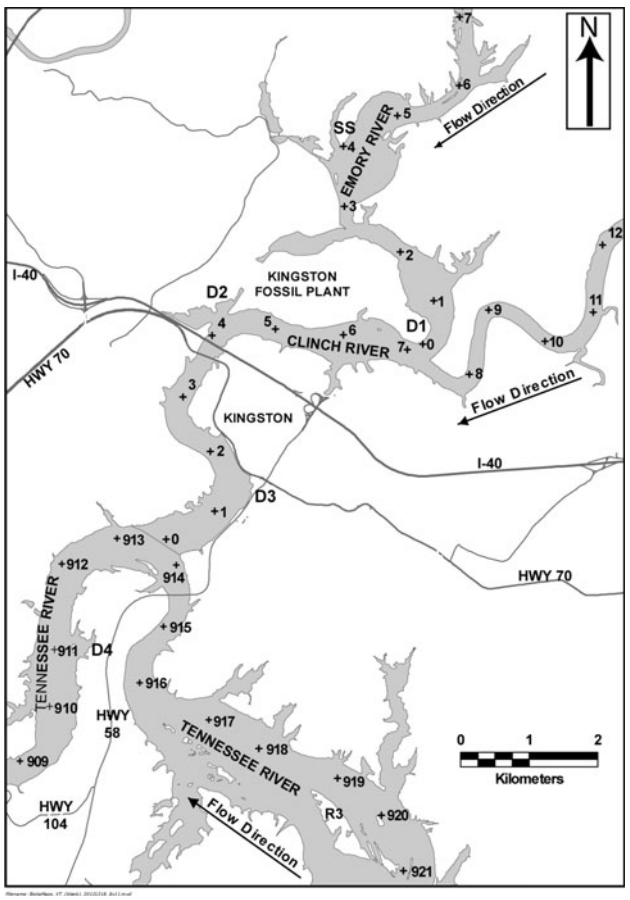

Fig. 1 Tree swallow colonies located near Kingston, TN. The study area consisted of two colonies located near the fly ash spill, one at the spill site (*SS*) and a second [downstream 1 (*D1*)] located at the confluence of the Emory and Clinch Rivers. Moderate-impacted colonies are located on the Clinch River at *D2* and *D3*, and a low-impacted colony (*D4*) is located downstream on the Tennessee River. Two reference colonies are located near Lenoir City, TN, at Ft. Loudoun Dam (*R1*) and Tellico Dam (*R2*) as well as a "positive control" located at Melton Hill Dam and are not shown here. A third reference colony is located on Long Island (*R3*) on the Tennessee River upstream from the confluence with the Clinch River. *Numbers* in rivers denote river kilometers

Tree swallows were present at the colonies in late February, 2011, and all nest boxes were available to swallows by the end of the first week of March. We placed all boxes within 70 m of the shoreline and 35 m apart when in a single row or 45 m apart and staggered when in a double row. Tree swallows initiated egg-laying in late April, and nestlings were present from mid-May through mid-July 2011. We collected food samples from tree swallows by trapping adults in the nest box immediately after they entered to provision young that were 3–13 days old (3- to 5-day-old nestlings $n = 26$, 6 to 9 days $n = 54$, 10–13 days $n = 27$, and unknown age $n = 2$). We attempted to capture adults for 1–2 h/day at each box from sunrise until 11 am to obtain bolus samples. We allowed 48 h to pass between trapping attempts and typically obtained one bolus sample per box but occasionally obtained two, one from each sex and typically on different days. Tree swallows bred synchronously and thus most of the bolus samples were collected in May ($n = 86$), but additional samples were collected in June ($n = 17$) and July ($n = 6$) in an attempt to account for seasonal changes in insect availability. When trapped, adult birds continued to hold the food bolus in their bill, and we used tweezers to remove the sample and place it in a sterile collection bag. Bolus samples were stored in a cooler or refrigerator for up to 8 h. We transferred each bolus sample into a preweighed 1.5-ml Eppendorf tube (New York, NY) and obtained the

sample wet weight to the nearest 0.1 mg using an analytical balance. Original sample wet masses ranged from 1.7 to 328.9 mg (mean 83.9 ± 6.82). Samples were stored at −20 °C until insect identification.

Insect Identification

To identify insects, bolus samples were thawed and gently pulled apart using tweezers and rinsing with Millipore water (Billerica, MA) in a sterile petri dish. Insects were viewed individually under a compound or dissecting microscope on a sterile glass slide and identified to order. Insects in the orders Diptera, Hymenoptera, Hemiptera, and Coleoptera were identified to family when diagnostic features were available. This was especially critical for Diptera and Coleoptera because only some families possessed an aquatic larval stage. We classified families as having an aquatic stage in their life cycle as long as some members of the family exhibited this characteristic and classified them as terrestrial when all members of the family lacked an aquatic stage in their life cycle. It was impossible for us to identify insects further than family due to sample degradation. The number of individuals in each order and family were tabulated as were the number of insects we were unable to identify. We were unable to obtain the biomass of each taxon in each bolus sample. However, we collected 5 bolus samples that contained only Chironomidae and in 2012 obtained 12 samples that contained only Ephemeroptera (unpublished data 2012). We used these samples to calculate the average dry mass of single midge (0.76 ± 0.39 mg) and mayfly (25.65 ± 5.85 mg) in our system. We then estimated the dry mass of these two taxa in all of the bolus samples by multiplying the average mass of a single individual by the number of individuals of that taxon in the bolus sample.

Trace Element Analysis

We prepared samples for trace element analysis by placing each bolus in a preweighed metal-free falcon tube (VWR Scientific, Radnor, PA). The samples were covered with a Kimwipe (Kimtech, Roswell, GA) secured with a rubber band and placed in a −80 °C freezer for at least 20 min before being placed in a freeze drier. Approximately 20 of the samples were reweighed at 24-h intervals to determine when they were dry based on mass equilibration of the sample. Samples were shipped overnight on dry ice for analysis at the Trace Element Analysis Laboratory at Dartmouth College.

Samples were digested in the metal-free falcon tubes by open vessel digestion with 0.5 ml 9:1 HNO_3:HCl (Optima, Fisher Scientific, St Louis MO) using microwave heating at 105 °C for 45 min. After cooling, 0.1 ml H_2O_2 was added to the samples, and the microwave heating step was repeated. The samples were then diluted to 10 ml with deionized water. The digested samples were analyzed for trace element concentrations by collision cell inductively coupled plasma–mass spectrometry (7700x; Agilent, Santa Clara, CA). Concentrations of As, barium (Ba), Cd, Cr, Cu, iron (Fe), manganese (Mn), strontium (Sr), thallium (Tl), vanadium (V), and zinc (Zn), (helium collision mode), Se (reaction mode), and Hg (normal mode) were quantified in each sample. Digestion quality-control measures included digestion blanks, fortified blanks, and reference materials at a frequency of 1 each/20 samples. There was insufficient bolus material to allow for digestion of duplicates or spikes. Analytical sample duplicates and spikes were performed at a frequency of 1 each/20 samples. Additional quality control consisted of reporting limit checks, interference checks, and initial and continuing calibration checks and blanks.

Detection limits for each bolus sample varied because the mass of each bolus sample used in the analysis varied. If the trace element concentration was lower than the detection limit, we assigned that sample a concentration of half the detection limit for statistical comparisons. The average detection limits (μg/g dry mass) for each element were as follows: As = 0.014 μg/g, Ba = 0.035 μg/g, Cd = 0.007 μg/g, Cr = 0.415 μg/g, Cu = 0.207 μg/g, Fe = 6.916 μg/g, Mn = 0.069 μg/g, Hg = 0.138 μg/g, Se = 0.207 μg/g, Sr = 0.027 μg/g, Tl = 0.007 μg/g, V = 0.014 μg/g, and Zn = 1.38 μg/g. Trace elements had to be present above the detection limit in >50 % of the samples at two of the colonies to be included in statistical models. Cr and Hg concentrations were lower than the detection limit in more than half of the samples from each colony and were not considered further. Average relative % difference for all 13 trace elements over 6 analysis duplicates was 12 ± 2 %. Average % recovery for 13 trace elements over 5 analysis spiked samples was 97 ± 21 %. Average % recovery for As, Cd, Cu, Fe, Mn, Hg, Se, Sr, and Zn was 100 ± 13 % for 5 separate digestions of National Institute of Standards and Technology (NIST) 2976, and Cr recovery averaged 48 % presumably because the Cr was in a form that is not solubilized by the open vessel acid digestion used here. Other elements analyzed in the bolus samples were not certified in the NIST standard.

Analysis

We tallied the number of each taxon that was collected and the number of times a taxon was present in a bolus sample across the entire study area. This was used to determine what proportion of the tree swallow diet consisted of each taxon and how frequently that taxon was consumed across the entire study area. We also calculated the overall percentage of aquatic insects present in each bolus sample out

Table 1 Composition of 109 bolus samples collected from tree swallows as they provisioned their young

Order Family	Arthropods (*n*)	Arthropods (%)	Bolus samples taxon present (*n*)	Bolus samples taxon present (%)
Diptera (flies)	**2,853**	**77.63**	**99**	**90.82**
Unknown diptera	284		59	
Agromyzidae (lead miner flies) (T)	5		4	
Anisopodidae (wood gnats) (T)	5		4	
Bibionidae (March flies) (T)	**44**	**1.20**	**3**	**2.75**
Calliphoridae (blow flies) (T)	2		2	
Ceratopogonidae (biting midges) (A)	3		2	
Cecidomyiidae (gall midges) (T)	4		3	
Chironomidae (midges) (A)	**2,187**	**59.51**	**71**	**65.14**
Chloropidae (fruit flies) (T)	14		9	
Culicidae (mosquitoes) (A)	**21**	**<1**	**13**	**11.93**
Dolichopodidae (long-legged flies) (A)	**17**	**<1**	**12**	**11.01**
Drosophilidae (vinegar flies) (T)	13		5	
Empididae (dance flies) (A)	**27**	**<1**	**18**	**16.51**
Ephydridae (shore flies) (A)	3		2	
Milichiidae (freeloader flies) (T)	6		3	
Muscidae (house flies) (A)	5		5	
Mycetophilidae (fungus gnats) (T)	**15**	**<1**	**14**	**12.85**
Phoridae (scuttle flies) (A)	**14**	**<1**	**11**	**10.09**
Platystomatidae (signal flies) (T)	1		1	
Sarcophagidae (flesh flies) (A)	6		6	
Scatopsidae (dung midges) (T)	4		3	
Scathophagidae (dung flies) (A)	3		1	
Sciaridae (dark-winged fungus gnats) (T)	4		4	
Sepsidae (black scavenger flies) (T)	9		7	
Simuliidae (black flies) (A)	**81**	**2.20**	**20**	**18.35**
Sphaeroceridae (lesser dung flies) (T)	7		4	
Stratiomyidae (soldier flies) (A)	22		10	
Syrphidae (flower flies) (A)	**31**	**<1**	**16**	**14.68**
Tachinidae (tachinid flies) (T)	9		9	
Tephritidae (fruit flies) (T)	2		2	
Tipulidae (crane flies) (A)	2		2	
Xylomyidae (wood soldier flies) (T)	1		1	
Xylophagidae (awl flies) (T)	2		1	
Hymenoptera (wasps, bees, ants)	**237**	**6.45**	**46**	**42.20**
Unknown Hymenoptera	26		19	
Braconidae (Braconid parasitoid wasps) (T)	19		9	
Chalcidoidea (Chalcid parasitoid wasps) (T)	4		4	
Diapriidae (Diaprid parasitoid wasps) (T)	1		1	
Formicidae (ants) (T)	**141**	**3.84**	**22**	**20.18**
Ichneumonidae (Ichneumon parasitoid wasps) (T)	11		8	
Scelionidae (parasitoid wasps) (T)	34		4	
Tenthredinidae (common sawflies) (T)	1		1	
Hemiptera (true bugs & leaf hoppers)	**388**	**10.56**	**66**	**60.55**
Unknown Hemiptera	4		4	
Aphididae (aphids) (T)	**239**	**6.50**	**44**	**40.37**
Cercopidae (froghoppers) (T)	**76**	**2.07**	**18**	**16.51**
Cicadellidae (leafhopper) (T)	**42**	**1.14**	**25**	**22.94**

Table 1 continued

Order Family	Arthropods (*n*)	Arthropods (%)	Bolus samples taxon present (*n*)	Bolus samples taxon present (%)
Delphacidae (planthoppers) (T)	1		1	
Flatidae (flatid planthoppers) (T)	1		1	
Membracidae (treehopper) (T)	1		1	
Miridae (leaf bugs) (T)	9		7	
Psyllidae (jumping plant lice) (T)	13		9	
Saldidae (shore bugs) (T)	1		1	
Tingidae (lace bugs) (T)	1		1	
Ephemeroptera (mayflies) (A)	13	<1	7	6.42
Isoptera (termites) (T)	**41**	**1.12**	**5**	**4.59**
Plecoptera (stoneflies) (A)	1	<1	1	<1
Psocoptera (barkflies) (T)	5	<1	4	3.67
Lepidoptera (moths & butterflies) (T)	8	<1	6	5.51
Coleoptera (beetles)	**57**	**1.55**	**26**	**23.85**
Unknown Coleoptera	12		9	
Carabidae (ground beetle) (T)	2		2	
Chrysomelidae (leaf beetle) (T)	1		1	
Ciidae (minute tree fungus beetle) (T)	1		1	
Curculionidae (snout beetles) (T)	16		8	
Elateridae (click beetles) (T)	1		1	
Histeridae (clown beetles) (A)	7		3	
Hydrophilidae (water scavenger beetles) (A)	1		1	
Mordellidae (tumbling flower beetles) (T)	1		1	
Rhipiphoridae (wedge-shaped beetles) (T)	1		1	
Scarabaeidae (scarab beetles) (T)	3		2	
Scolytidae (bark beetle) (T)	6		2	
Staphylinidae (rove beetles) (T)	4		4	
Tenebrionidae (darkling beetles) (T)	1		1	
Tricoptera (caddisflies) (A)	**29**	**<1**	**15**	**13.76**
Araneae (spiders) (T)	5	<1	4	3.67
Total arthropods	3,675			

The common name of the taxon is given in parentheses next to the scientific name. Taxa with an aquatic stage in their life cycle are denoted by an A and those with a strictly terrestrial life cycle by a T. Taxa in bold comprised >1 % of the total arthropods sampled and/or were present in >10 % of the bolus samples collected. Unknown denotes individuals identified to order but that could not be accurately identified as to family

of the total number of insects that were classified as aquatic or terrestrial in the sample. We examined the effects of nestling age (number of days posthatch) across nests on bolus composition using correlations. The effect of season on bolus composition was evaluated by correlating Julian date of sample collection with bolus composition.

To examine diet composition among colonies, we limited our analysis to locations where we collected a minimum of four bolus samples. We obtained only three bolus samples from reference 3 and had to exclude this colony from the analysis. For each bolus sample, we calculated the proportion of insects with an aquatic life stage and the proportion of the sample that consisted of the following groups—non-Chironomidae Diptera, Chironomidae, Hymenoptera, Hemiptera, and Coleoptera—as well as the proportion of other taxa out of the total number of insects present in each sample. We selected these taxa because they appeared to be an important part of the tree swallow diet based on their occurrence in bolus samples (Table 1). Because these proportions are not independent of each other and must sum to one, we used compositional analysis to produce five new variables that were linearly independent of each other (Aitchison 1986). We did this by log-transforming the quotient produced by dividing the proportion of a sample that consisted of a taxon of interest by the proportion of that sample classified as other. In cases where the proportion was 0 (and therefore could not be log-transformed), we added 0.005 to the sample proportion, which was an order of magnitude smaller than our smallest proportion (0.015). The log-transformed variables were

used to compare diet composition among colonies (see later text).

We log transformed all element concentrations because the data were not normally distributed. All of the trace elements examined here are associated with coal fly ash (Rowe et al. 2002) and we expected *a priori* that trace element concentrations would be highly correlated. To decrease the dimensionality of the data set, we used principal components analysis (PCA) to produce three principal components of trace element concentrations. We performed multivariate analyses of variance to compare principal components (PC) scores or bolus composition among colonies. We included nestling age and Julian sample collection date as covariates in the model, but removed them from the final model if $p > 0.10$, and checked that these covariates did not violate the assumption of slope homogeneity. To determine if trace element exposure varied with the composition of the diet, we performed three backward linear regressions with the transformed prey composition proportions as independent variables and the PCs of log-transformed trace element concentrations as the dependent variables. Variables were removed from the model if $p > 0.10$. We also performed regressions between the PC scores and the dry mass of Chironomidae and Chironomidae plus Ephemeroptera in the samples to determine if the results were similar to those produced using the proportion data. Trace element concentrations were quantified for entire bolus samples, not individual taxa, and we were thus unable to compare trace element concentrations among taxa. All statistical tests were two-tailed, and we set $\alpha = 0.05$. All statistical analyses were performed using PASW Statistics GradPack 18 (SPSS, Chicago, IL).

Results

Composition of Bolus Samples and Temporal Changes in Diet

We collected 109 bolus samples across the study area, and samples ranged in size from a single insect to 170 insects [average number of insects per bolus = 33.7 ± 3.4 (median = 23)]. We were able to classify 3,675 arthropods to order and were unable to identify 38 due to degradation. Of the 3,675 arthropods classified, 2,473 (67.3 %) had an aquatic stage in their life cycle representing a large proportion of the tree swallow diet. The average percent biomass of Chironomidae and Ephemeroptera combined in bolus samples was 63.5 ± 11.4 % of total sample dry mass. The combined Chironomidae and Ephemeroptera biomass significantly positively correlated with the proportion of the bolus that consisted of aquatic insects ($r^2 = 0.236$, $F_{1,\ 106} = 32.79$, $p < 0.001$). The tree swallow diet consisted primarily of dipterans; this order constituted 77.6 % ($n = 2{,}853$) of the total number of insects collected and was present in 99 of the 109 (90.8 %) bolus samples (Table 1). The family Chironomidae, which includes members with an aquatic larval stage, made up 59.5 % ($n = 2{,}187$) of the total arthropods collected in bolus samples and was present in 65.1 % of the bolus samples collected ($n = 71$). The average dry mass of Chironomidae in the bolus samples was 65.2 ± 10.5 % of total sample dry mass. The dry mass of Chironomidae also correlated well with the numerical proportion of Chironomidae in the sample ($r^2 = 0.590$, $F_{1,\ 106} = 152.4$, $p < 0.001$). Several other dipteran families that have an aquatic stage in their life cycle appeared in >10 % of the bolus samples collected and included the families Culicidae, Dolichopodidae, Empididae, Phoridae, Simuliidae, and Syrphidae. The orders Hymenoptera, Hemiptera, Coleoptera, and Trichoptera were present in at least 13.8 % of bolus samples, but only trichopterans (caddisflies) and some coleopteran families have an aquatic larval stage. However, the coleopteran families with an aquatic larval stage represented a small proportion of insects consumed by the swallows (Table 1). We focused subsequent analyses on proportions of non-Chironomidae Diptera, Chironomidae, Hemiptera, Hymenoptera, and Coleoptera because each of these taxa was present in >20 % of the bolus samples and represented a large part of the tree swallow diet.

We examined seasonal- and age-related changes in diet by performing correlations between these variables and the proportion of the diet that consisted of the five taxa predominantly consumed by tree swallows. Bolus samples collected later in the season had a greater proportion of Hymenoptera ($r = 0.294$, $n = 108$, $p = 0.002$), but the proportions of non-Chironomidae Diptera, Chironomidae, Hemiptera, and Coleoptera did not change with season (all $p \geq 0.49$). We also examined the consumption of Ephemeroptera, and it did not change seasonally ($p = 0.23$). Bolus composition changed with nestling age with the proportion of the diet that consisted of non-Chironomidae Diptera ($r = -0.324$, $n = 106$, $p = 0.001$), Hymenoptera ($r = -0.193$, $n = 106$, $p = 0.05$), and Coleoptera ($r = -0.202$, $n = 106$, $p = 0.04$) decreased with nestling age. The proportion of the diet consisting of Chironomidae and Hemiptera remained nearly constant with nestling age ($p \geq 0.56$).

Colony Effects on Bolus Composition

We examined the influence of colony location on the composition of bolus samples collected and included nestling age and sample collection date as covariates. Location significantly influenced the proportion of the diet

that consisted of Chironomidae ($F_{7,\ 97} = 3.556$, $p = 0.002$, Fig. 2) and Hemiptera ($F_{7,\ 97} = 2.808$, $p = 0.01$) with nearly significant differences for non-Chironomidae dipterans ($F_{7,\ 97} = 1.926$, $p = 0.07$) and coleopterans ($F_{7,\ 97} = 1.840$, $p = 0.09$). Nestling age affected the consumption of non-Chironomidae Diptera ($F_{1,\ 93} = 8.044$, $p = 0.006$), and sample collection date affected the consumption of Hymenoptera ($F_{1,\ 93} = 9.846$, $p = 0.002$). *Post hoc* tests showed that colony-related differences were due to the increased consumption of Chironomidae at the spill site compared with downstream 3 ($p = 0.014$) and downstream 4 ($p = 0.031$), with some evidence of greater consumption of Chironomidae at Melton Hill Dam than at downstream 3 ($p = 0.078$). Significantly fewer Hemiptera were consumed at downstream 3 than at reference 1 ($p = 0.027$) or downstream 1 ($p = 0.039$). We found no significant differences among colonies in the proportion of Hymenoptera consumed ($p = 0.587$). We also found that the proportion of insects with an aquatic larval stage consumed by tree swallows differed significantly among colonies ($F_{7,\ 97} = 3.921$, $p = 0.001$, Fig. 3). A greater proportion of insects with an aquatic stage in their life cycle were consumed at Melton Hill Dam compared with downstream 3 ($p = 0.005$), downstream 4 ($p = 0.010$), and reference 1 ($p = 0.087$). More insects with an aquatic larval stage were consumed at the spill site compared with downstream 3 ($p = 0.036$) and downstream 4 ($p = 0.054$).

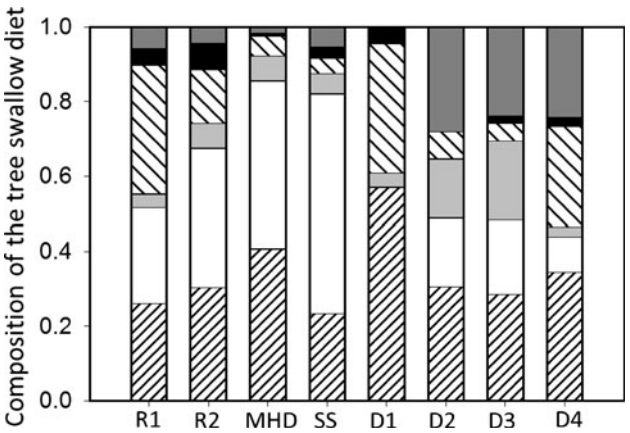

Fig. 2 Composition of bolus samples across the study area. All statistical analyses were performed on transformed data, but we present untransformed proportions in graphs for clarity. We found that location significantly influenced the proportion of the diet that consisted of Chironomidae ($F_{7,\ 97} = 3.556$, $p = 0.002$) and Hemiptera ($F_{7,\ 97} = 2.808$, $p = 0.010$). A greater proportion of the diet consisted of Chironomidae at the spill site compared with downstream 3 (*D3*) and *D4* (all $p \leq 0.031$). A smaller proportion of the diet consisted of Hemiptera at *D3* compared with reference 1 and *D1* (all $p \leq 0.039$). Categories included non-Chironomidae Diptera (*narrow diagonal*), Chironomidae (*white*), Hymenoptera (*light gray*), Hemiptera (*broad diagonal*), Coleoptera (*black*), and other (*dark gray*)

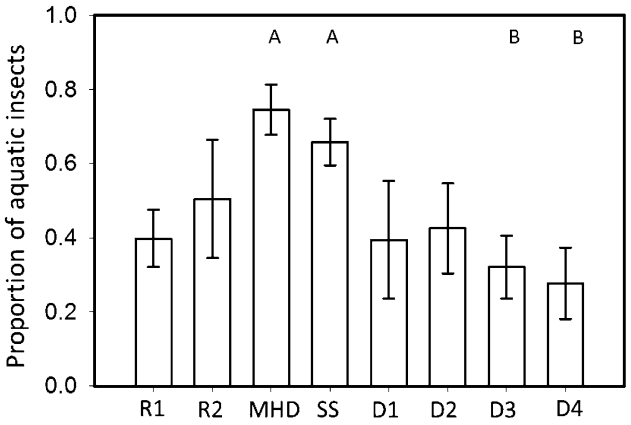

Fig. 3 Proportion of the tree swallow diet consisting of insects with an aquatic stage in their life cycle by colony. The proportion of insects with an aquatic stage in their life cycle consumed by tree swallows differed significantly among colonies ($F_{7,\ 97} = 3.921$, $p = 0.001$). Different *letters* above *bars* indicate colonies that were significantly different in *post hoc* comparisons ($p \leq 0.010$); *bars* with the *same letter* or *no letter* do not differ significantly. *Error bars* represent SEs around the mean

Colony and Diet Effects on Trace Element Concentrations

Principal components analysis (PCA) produced three PCs that together explained 64.5 % of the variance in trace element concentrations (Table 2). PC1 received high, positive factor loadings for As, Ba, Cd, Cu, Fe, Mn, Sr, Se, and Zn and explained 36.1 % of the variance in trace element concentrations. PC2 received high, positive factor loadings for As and V, whereas PC3 had high, positive loadings for Se and Tl. PC2 explained an additional 18.2 %

Table 2 Factor loadings for PCA analysis of trace element concentrations

Trace element	PC1	PC2	PC3
As	0.548	0.644	0.189
Ba	0.557	0.161	−0.594
Cd	0.675	−0.183	−0.025
Cu	0.568	−0.454	−0.024
Fe	0.819	−0.170	0.198
Mn	0.581	−0.458	−0.184
Se	0.697	0.156	0.422
Sr	0.763	0.377	−0.209
Tl	0.022	0.054	0.651
V	0.365	0.777	−0.059
Zn	0.607	−0.555	0.124
Eigen value	3.966	2.001	1.128
Variance (%)	36.07	18.19	10.26

Trace element concentrations were log-transformed to improve normality before performing PCA

of the variance and PC3 an additional 10.3 % of the variance in trace element concentrations.

We found significant differences among colonies in PC1 ($F_{7,\ 96} = 3.343$, $p = 0.003$) and PC3 ($F_{7,\ 96} = 2.269$, $p = 0.035$) scores, whereas PC2 scores did not differ among colonies ($p = 0.127$, Fig. 4). These results indicated that greater concentrations of some trace elements, particularly Fe, Se, and Sr, were present in bolus samples at the spill site than at the other colonies (Table 3). For PC1, *post hoc* tests showed that the spill site had significantly higher PC1 scores than reference 1 ($p = 0.022$), downstream 2 ($p = 0.027$), and downstream 4 ($p = 0.019$), and suggested a trend for PC1 scores to be greater than those at downstream 3 ($p = 0.075$). PC3 scores tended to be lower at Melton Hill than at the spill site ($p = 0.074$) and downstream 4 ($p = 0.100$). However, concentrations of most trace elements were lower than levels of toxicological concern (Table 3).

The proportion of the diet that consisted of aquatic insects was positively related to the exposure of tree swallows to trace elements associated with PC1 ($r^2 = 0.190$, $F_{1,\ 104} = 24.38$, $p < 0.001$, Fig. 5a). The proportion of the diet that consisted of aquatic insects was unrelated to exposure to trace elements associated with PC2 and PC3 ($p \geq 0.417$). When we focused on diet composition, exposure to trace elements associated with PC1 was best explained by a model that included the proportion of Chironomidae and Hemiptera in the diet (Fig. 5b, c, $r^2 = 0.374$, $F_{2,\ 103} = 30.82$, $p < 0.001$). Examining each taxon separately, we found that consumption of Chironomidae was positively related to trace element exposure ($r^2 = 0.338$, $F_{1,\ 104} = 53.21$, $p < 0.001$), whereas consumption of Hemiptera was weakly negatively related to exposure ($r^2 = -0.041$, $F_{1,\ 104} = 4.415$, $p = 0.038$). PC1 scores were also positively related to the dry mass of Chironomidae in the bolus samples

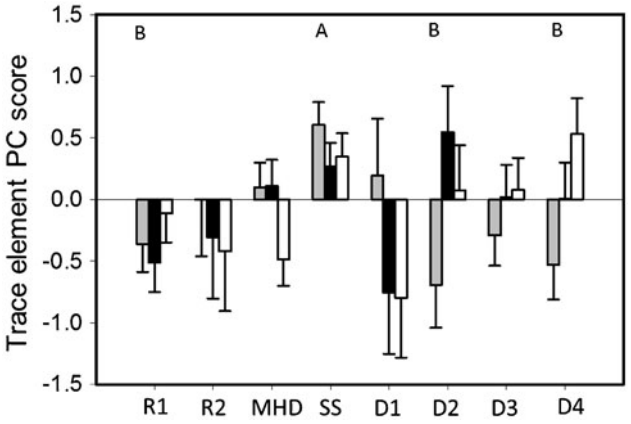

Fig. 4 PCs of trace element concentrations in tree swallow bolus samples by colony: PC1 (*gray*), PC2 (*black*), and PC3 (*open*). We found significant differences among colonies in PC1 ($F_{7,\ 96} = 3.359$, $p = 0.003$) and PC3 ($F_{7,\ 96} = 2.977$, $p = 0.007$), but PC2 did not differ among colonies ($p = 0.109$). *Letters* above *bars* denote significant differences among colonies for PC1 scores (all $p \leq 0.028$) such that colonies with *different letters* differ significantly from each other, whereas *bars* with *no letter* or the *same letter* do not differ significantly. *Post hoc* analyses showed trends for Melton Hill Dam to have lower PC3 scores than the spill site ($p = 0.074$) and D4 ($p = 0.100$)

($r^2 = 0.160$, $F_{1,\ 104} = 19.84$, $p < 0.001$) and to the combined mass of Chironomidae and Ephemeroptera in the bolus samples ($r^2 = 0.145$, $F_{1,\ 104} = 17.58$, $p < 0.001$). For PC2, we found a weak but nearly significant negative relationship between the proportion of Hymenoptera in the bolus sample and PC2 scores ($r^2 = -0.031$, $F_{1,\ 104} = 3.377$, $p = 0.069$). The mass of Chironomidae and the combined Chironomidae and Ephemeroptera mass were unrelated to PC2 scores ($p \geq 0.534$). PC3 scores were best explained by a model that contained the proportion of non-Chironomidae Diptera and Chironomidae ($r^2 = 0.136$, $F_{1,\ 104} = 8.111$, $p = 0.001$). Examining each taxon separately, the

Table 3 Univariate means and SEs of trace element concentrations in tree swallow bolus samples by location (μg/g dry mass)

E	Reference 1	Reference 2	Melton Hill Dam	Spill site	Downstream 1	Downstream 2	Downstream 3	Downstream 4
As	0.07 ± 0.03	0.10 ± 0.03	0.17 ± 0.07	0.24 ± 0.07	0.04 ± 0.02	0.10 ± 0.03	0.13 ± 0.02	0.10 ± 0.04
Ba	2.1 ± 0.3	4.7 ± 1.4	13.3 ± 3.8	8.0 ± 3.2	15.8 ± 9.6	3.19 ± 1.2	12.25 ± 10.5	4.46 ± 2.2
Cd	0.27 ± 0.05	0.40 ± 0.11	0.40 ± 0.05	0.50 ± 0.09	0.66 ± 0.49	0.24 ± 0.09	0.57 ± 0.25	0.29 ± 0.12
Cu	19.7 ± 2.4	24.2 ± 10.0	19.9 ± 2.9	19.6 ± 2.1	28.1 ± 13.1	15.9 ± 7.5	14.9 ± 1.7	12.7 ± 2.6
Fe	186.0 ± 50.9	192.5 ± 64.3	130.5 ± 18.8	380.4 ± 49.6	138.6 ± 41.7	85.8 ± 23.0	139.8 ± 38.6	120.5 ± 26.5
Mn	73.0 ± 37.8	48.2 ± 22.0	44.2 ± 8.2	37.4 ± 4.7	42.7 ± 13.5	15.0 ± 5.3	29.6 ± 7.5	47.5 ± 20.1
Se	0.63 ± 0.23	0.65 ± 0.27	2.07 ± 0.54	3.07 ± 0.39	0.67 ± 0.32	1.51 ± 0.74	1.23 ± 0.29	0.67 ± 0.18
Sr	1.7 ± 0.5	3.2 ± 0.8	4.8 ± 1.0	17.0 ± 11.4	3.3 ± 1.3	3.4 ± 1.5	3.1 ± 1.3	2.5 ± 1.0
Tl	0.01 ± 0.006	0.01 ± 0.002	0.01 ± 0.002	0.02 ± 0.003	0.01 ± 0.001	0.02 ± 0.011	0.01 ± 0.008	0.10 ± 0.070
V	0.01 ± 0.003	0.02 ± 0.003	0.05 ± 0.026	0.20 ± 0.151	0.01 ± 0.002	0.05 ± 0.031	0.05 ± 0.027	0.02 ± 0.012
Zn	122.8 ± 14.7	127.5 ± 24.9	111.1 ± 10.5	113.6 ± 9.74	257.6 ± 118.7	70.8 ± 17.4	112.7 ± 14.7	114.8 ± 22.2
n	17	4	21	26	4	7	14	11

n number of samples collected from each colony, *E* element

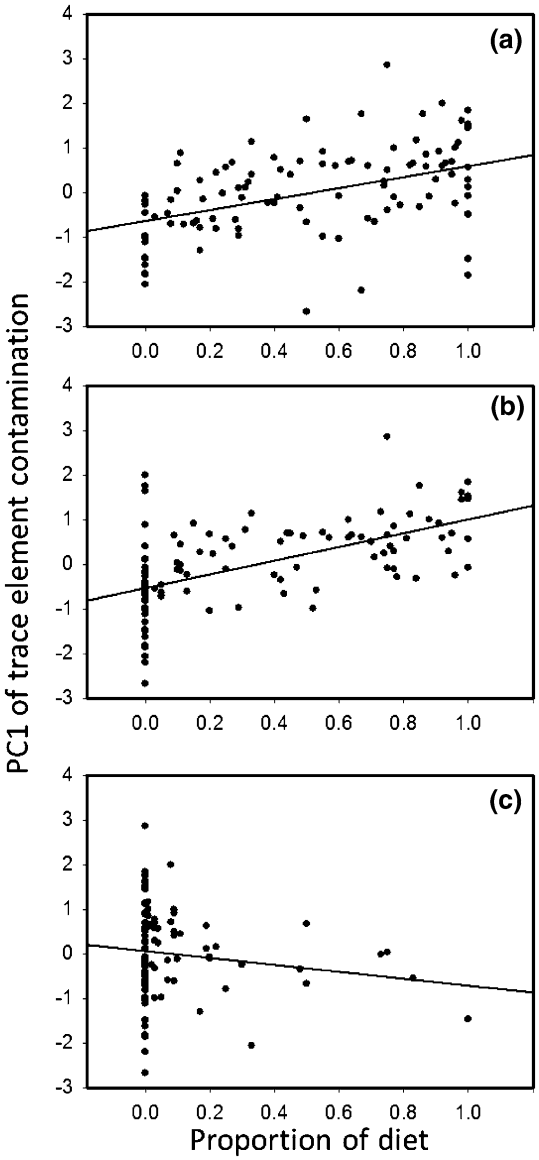

Fig. 5 Exposure to trace element contaminants is affected by composition of the tree swallow diet. Tree swallows that consumed a greater proportion of **a** aquatic insects ($r^2 = 0.191$, $p < 0.001$) and **b** Chironomidae ($r^2 = 0.338$, $p < 0.001$) were exposed to greater concentrations of trace elements associated with PC1. Consumption of Hemiptera **c** was negatively related to exposure to PC1 contaminants ($r^2 = -0.041$, $p = 0.038$). All statistical analyses were performed on transformed data, but we present untransformed values in graphs for clarity

consumption of non-Chironomidae Diptera was negatively related to PC3 scores ($r^2 = -0.059$, $F_{1,\ 104} = 6.571$, $p = 0.012$), and PC3 scores tended to increase as consumption of Chironomidae increased ($r^2 = 0.023$, $F_{1,\ 104} = 2.450$, $p = 0.121$). The combined dry mass of Chironomidae and Ephemeroptera in bolus samples was weakly, positively related to PC3 scores ($r^2 = 0.042$, $F_{1,\ 104} = 4.508$, $p = 0.038$), but the mass of Chironomidae alone was unrelated to PC3 scores ($p = 0.203$).

Discussion

We examined the diet of tree swallows to determine whether trace elements from a recently remediated coal fly ash spill were entering the terrestrial ecosystem through the consumption of emerging aquatic insects. Across the entire study area, more than half of the tree swallow diet consisted of insects that had an aquatic stage in their life cycle. Dipterans, particularly members of the family Chironomidae, which have an aquatic larval stage, were consumed in the largest quantities by tree swallows. Hymenopterans, hemipterans, and coleopterans were also brought to young in appreciable quantities. Thus, looking at our results across the entire study area, it appears that tree swallows have the capacity to be exposed to trace elements through the consumption of insects with an aquatic larval stage, particularly due to the consumption of Chironomidae. However, we found significant differences among colonies in the proportion of the diet that consisted of insects with an aquatic stage in their life cycle and of Chironomidae. Tree swallows at the spill site and Melton Hill Dam consumed more insects with an aquatic larval stage, and this led birds at the spill site to be exposed to more aquatic trace elements from the fly ash spill.

A greater proportion of the diet consisted of Hemiptera at downstream 1 and reference 1 compared with the other colonies. This dietary difference is likely due to naturally occurring habitat differences because reference 1 and downstream 1 are located near large fields with deep grass, whereas Melton Hill Dam and the spill site are essentially well-maintained lawns that may provide poor habitat for terrestrial insects. Reference 1 may provide poor aquatic habitat for insects as well because this site is adjacent to a marina. The lack of consumption of Chironomidae at downstream 1 is odd given that surveys of nearby sections of the Emory River detected numerous Chironomidae larvae (Baker 2011). It is unclear what factors may have limited the consumption of Chironomidae at this colony or if it is an artifact of the small number of bolus samples collected here. Aquatic substrate, habitat heterogeneity, water depth, water temperature, pollution, and metal tolerance can affect aquatic insect diversity and could explain the differences detected between Melton Hill Dam, reference 1, downstream 1, and the spill site (Clements et al. 1992; Kasangaki et al. 2006; Wright and Burgin 2009; Odume and Muller 2011; Rosa et al. 2011). In the future, it would be worthwhile to better quantify habitat characteristics to determine how they affect insect availability.

We found few seasonal changes in the tree swallow diet. The proportion of the diet consisting of Hymenoptera increased later in the season, but the consumption of other taxa did not change seasonally. Although Ephemeroptera were not consumed in large quantities in this study, other

studies have indicated that they can be an important component of the tree swallow diet and that their consumption increases seasonally and can be affected by weather conditions (Smits et al. 2005; Papp et al. 2007). At our study site, many tree swallows had old nestlings or had fledged their young before most mayflies emerged in late May (personal observation 2011), and this may have limited mayfly consumption or our ability to obtain bolus samples containing mayflies. It is possible in this system that greater numbers of Ephemeroptera would be consumed in a year when mayfly hatches better coincide with tree swallow nesting phenology.

We also found that nestling age significantly affected the proportion of the diet that consisted of non-Chironomidae Diptera, Hymenoptera, and Coleoptera. In all cases, the proportion of these taxa in the diet decreased with nestling age. However, other studies on tree swallows have detected little change in diet with nestling age (McCarty and Winkler 1999; Bortolotti et al. 2011). It is possible that the changes we detected were the result of short-term variation in the availability of prey that were not detected by our seasonal analysis. It is unlikely that this variation in the diet would lead to age-specific exposure to trace elements because the proportion of the diet consisting of Chironomidae remained fairly constant with nestling age. This taxon comprised a major portion of tree swallow diet and could be the primary route of trace element exposure to tree swallows in this system.

Bolus samples from swallows at the spill site had significantly higher PC1 scores than many of the reference and downstream colonies. This indicates that birds at the spill site are exposed to greater concentrations of some trace elements, including Se, Sr, and Fe, than birds at other colonies. The spill site also tended to have significantly higher PC3 scores than Melton Hill Dam, as did downstream 4, but these differences were not statistically significant. Increased PC3 scores at downstream 4 could be related to residual contamination from the spill or the result of other sources of contamination upstream on the Tennessee River. Reference 3 is located a few km upstream from downstream 4 on the Tennessee River, but we were unable to examine bolus trace element concentrations at reference 3 due to a small sample size. Thus, we were unable to determine if increased concentrations of trace elements were present in the tree swallow diet in nearby portions of the Tennessee River or were found only in the vicinity of the spill.

One of the most important findings of our study showed the importance of aquatic insects as vectors of contamination to terrestrial consumers. Tree swallows whose diet consisted predominantly of insects with an aquatic stage in their life cycle were exposed to higher levels of trace elements associated with PC1. In particular, the consumption of Chironomidae led to greater exposure to trace elements, whereas consumption of Hemiptera and Hymenoptera was negatively related to trace element exposure. Using the mass of Chironomidae or the combined mass of Chironomidae and Ephemeroptera also indicated that greater consumption of these taxa was associated with exposure to trace elements associated with PC1 and/or PC3. Although tree swallows consuming insects with an aquatic larval stage were exposed to greater concentrations of trace elements, these dietary concentrations were lower than levels typically associated with adverse reproductive effects. The highest dietary concentrations of Se in our study occurred at the spill site and were at the lower limit of the dietary concentration range thought to result in reproductive effects in avian species (Ohlendorf and Heinz 2011). Most of the trace element concentrations detected in tree swallow bolus samples were an order of magnitude lower than concentrations detected in a study performed on common grackles (*Quiscalus quiscala*) breeding near an active ash settling basin (Bryan et al. 2012). Indeed, concentrations of many of the trace elements in our study were similar to other studies on tree swallows thst found no effects of trace element contamination on reproductive success or oxidative stress (Custer et al. 2006; Custer et al. 2008).

The results of our study show that emerging aquatic insects can transport trace elements to terrestrial consumers, such as tree swallows. This suggests that other terrestrial insectivores could be at risk of exposure to trace elements from coal fly ash. However, we detected low levels of trace elements in insects collected from the spill site. It is possible that this is a result of successful remediation efforts that occurred in the 2.5 years preceding our study and/or due to the dilution effects of a lotic environment. We also found that the tree swallow diet varied substantially in habitats that appeared superficially to be quite similar (*i.e.*, all open lawns or fields, limited tree cover, located near water). Despite locating boxes strategically within 70 m of the shore, tree swallows in some colonies foraged extensively on terrestrial insects, possibly limiting their exposure to aquatic trace elements. Our results suggest that in some systems it may be problematic to assume uniform consumption of particular types of prey or to infer exposure to aquatic contaminants solely by monitoring birds in riparian areas. We suggest that future studies examining the movement of aquatic contaminants into terrestrial consumers include dietary analysis to confidently document routes of exposure.

Acknowledgments We thank John Hallagan, Matthew Hepp, Dean Sedgwick, Mark Hepner, Elizabeth Burton, and Jesse Morris for assistance with field work. We also thank Dana Hawley for providing help in the field, advice, and logistical support. Jean Favara, Wes James, Suzy Young, Neil Carriker, and the rest of the TVA staff and

contractors at the Kingston site provided logistical support that was greatly appreciated. We thank Lisa Moore for identifying the insects and David Hankins for assisting with the map. James Van Dyke, David Steen, and Neil Carriker provided comments that greatly improved the quality of this manuscript. This research was funded by the TVA.

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