

## BIOACCUMULATION AND TROPHIC TRANSFER OF POLYCHLORINATED BIPHENYLS BY AQUATIC AND TERRESTRIAL INSECTS TO TREE SWALLOWS (*TACHYGINETA BICOLOR*)

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**Abstract**—Insectivorous passerines often bioaccumulate polychlorinated biphenyls (PCBs) via trophic transfer processes. Tree swallows (*Tachycineta bicolor*) frequently are used for estimating PCB bioaccumulation, yet the focus on specific trophic links between contaminated sediment and bird has been limited. Bioaccumulation of PCBs from sediment to tree swallows was examined with focus on trophic pathways by simultaneously examining PCBs in emergent aquatic and terrestrial insects and gut contents of nestlings. Total PCB concentrations increased from sediment ( $123.65 \pm 15.93 \mu\text{g}/\text{kg}$ ) to tree swallow nestlings ( $2,827.76 \pm 505.67 \mu\text{g}/\text{kg}$ ), with emergent aquatic insects, terrestrial insects, and gut content samples having intermediate concentrations. Biota–sediment accumulation factors (BSAFs) varied among congeners for tree swallow nestlings and for male and female *Chironomus* spp. For nestlings, the highest BSAF was for the mono-*ortho*-substituted congener 118. Nestling biomagnification values were similar for gut contents and female *Chironomus* spp., suggesting this diet item may be the main contributor to the overall PCB transfer to nestlings. However, gut content samples were highly variable and, on a PCB congener pattern basis, may have been influenced by other taxa, such as terrestrial insects. Considering dietary plasticity of many insectivorous birds, the present study suggests that a variety of potential food items should be considered when examining PCB accumulation in insectivorous passerines.

**Keywords**—Biota–sediment accumulation factors    Chironomidae    Polychlorinated biphenyls    Tree swallow    Trophic transfer

### INTRODUCTION

Lipophilic organic contaminants, such as polychlorinated biphenyls (PCBs), can bioaccumulate through a food web and, ultimately, confer a risk to higher-trophic-level organisms, such as insectivorous passerines. One such species, tree swallows (*Tachycineta bicolor*), frequently bioaccumulates PCBs from contaminated sediment through trophic transfer processes [1–4]. Although the proportion of PCBs in tree swallows compared to that in sediment may be used to assess the risk to wildlife at contaminated sites [2], the relationship may be heavily influenced by dynamics in trophic links between the two. It often is assumed that the trophic link between sediment and swallow consists primarily of emergent insects [1,2], because these insects have a sediment-associated larval stage and provide a path for transfer of PCBs from the aquatic to the terrestrial environment [5].

Previously, the role of emergent insects during PCB bioaccumulation in tree swallows has been examined with samples limited to a single emergence or broad taxonomic groupings [2,3,6], from which PCB magnification factors have been modeled for field-collected insects to tree swallows [6]. Tree swallows, however, are opportunistic, and both foraging activity and diet composition can shift in relation to the abundance of local prey items [7–9]. When the models of Nichols et al. [6] were tested using gut content samples that inherently considered these variables, magnification factors were roughly

doubled [1]. This may be attributed to interspecific variation in PCB exposure and uptake among insects coupled with plasticity in tree swallow diet, emphasizing the importance of a whole-diet assessment [1]. However, in Custer et al. [1], the specific prey taxa consumed by birds was unknown.

In the present study, PCB bioaccumulation from sediment to tree swallows was examined, with particular focus on elucidating the trophic pathways of PCB transfer by simultaneously examining PCBs in field-collected emergent aquatic and terrestrial insects at tree swallow nest sites and in gut contents of tree swallow nestlings. It is important to examine both emergent aquatic and terrestrial insects, because all diet items may influence PCB accumulation in tree swallows. Variation in diet breadth among other generalist predators may result in concentration-based shifts of PCB congener patterns [10], primarily because PCB concentrations often vary among the prey species of a top predator [11]. Thus, multiple bioaccumulation pathways need to be explored to understand better the trophic transfer processes occurring in the food web and, ultimately, for predicting contaminant risk to swallows and other insectivorous passerines.

The objective of the present study was to investigate trophic transfer and bioaccumulation patterns of individual PCB congeners from contaminated sediment to insects and tree swallows. Specific objectives included the following: Examining biota–sediment accumulation factors (BSAFs) and nestling biomagnification factors (BMFs) of individual congeners, total PCBs ( $\Sigma\text{PCB}$ ), and toxic equivalents (TEQs) relative to po-

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tential insect diet sources, and examining PCB congener patterns among a variety of potential insect diet items and nestling gut content samples to gain a better understanding of the trophic sources of PCBs in the diet of tree swallow nestlings.

## MATERIALS AND METHODS

### Study sites

Bioaccumulation and trophic transfer of PCBs in tree swallows was examined at the Sangamo Bay area of Crab Orchard Lake, a 2,820-ha reservoir located on the Crab Orchard National Wildlife Refuge, Williamson County, Illinois, USA (Fig. 1). By-products of electrical component production and PCBs, predominantly Aroclor 1254, were buried in the Area 9 landfill adjacent to Crab Orchard Lake at Sangamo Bay from 1946 to the mid-1960s [12,13]. Historically, PCB contamination has been reported to occur in the soil surrounding the landfill, lake sediment, groundwater, and wildlife species [12]. Crab Orchard Lake has been on the Superfund National Priorities List since 1987, and elevated PCB levels currently are detected in Sangamo Bay sediment. Additional historical information about the site has been reported previously [14]. A reference site was located approximately 13 km southwest of Crab Orchard Lake, along the spillway of Little Grassy Lake (Fig. 1). This site had no known history of PCB contamination.

### Sediment and insect collection

Sediment was collected at 15 locations based on historical data of contamination and recommendations of the U.S. Fish and Wildlife Service. Samples were collected using a 50-cm, liner-type, hand-core sampler (Wildco, Buffalo, NY, USA). Samples were stored at  $-4^{\circ}\text{C}$  pending PCB and total organic carbon (TOC) analyses. Only the uppermost 6-cm fraction of each sample was analyzed, with the lower fractions being discarded.

Emergent aquatic and terrestrial insects were collected for PCB analysis between June and July, concurrent with tree swallow sampling, using a picking-light method [15]. Specifically, a 15- to 22-W black light was suspended in front of a white piece of fabric ( $2.3 \times 2.3$  m) at a maximum distance of 3 m from the shoreline. Insects were removed from the fabric by aspiration into a sample jar. In the laboratory, insects were sorted into taxonomic groups, enumerated, and stored at  $-20^{\circ}\text{C}$  pending contaminant analysis. The initial focus for contaminant transfer included adult emergent aquatic insect taxa that had sediment-dwelling larval stages (e.g., midges [Chironomidae], mayflies [Ephemeroptera], and caddisflies [Trichoptera]). A large percentage of emergent insects collected during black-light sampling belonged to the family Chironomidae, with a majority of the biomass from the genera *Chironomus*. Thus, Chironomidae were differentiated into three groups: Female *Chironomus* spp., male *Chironomus* spp., and all remaining individuals within the family Chironomidae. All caddisflies collected belonged to the family Hydropsychidae and were analyzed as a group. Mayflies belonging to the genera *Callibaetis* and *Hexagenia* were collected in too few numbers for PCB analysis. In addition to emergent aquatic insects, taxa that were identified positively as occurring in tree swallow gut content samples (see below) also were analyzed for PCBs. Terrestrial insects were considered to be a group and included individual samples of terrestrial beetles (Chrysomelidae, Coccinellidae, Lampyridae, Carabidae, Silphidae, and Staphylinidae), leaf hoppers (Cicadellidae), ants

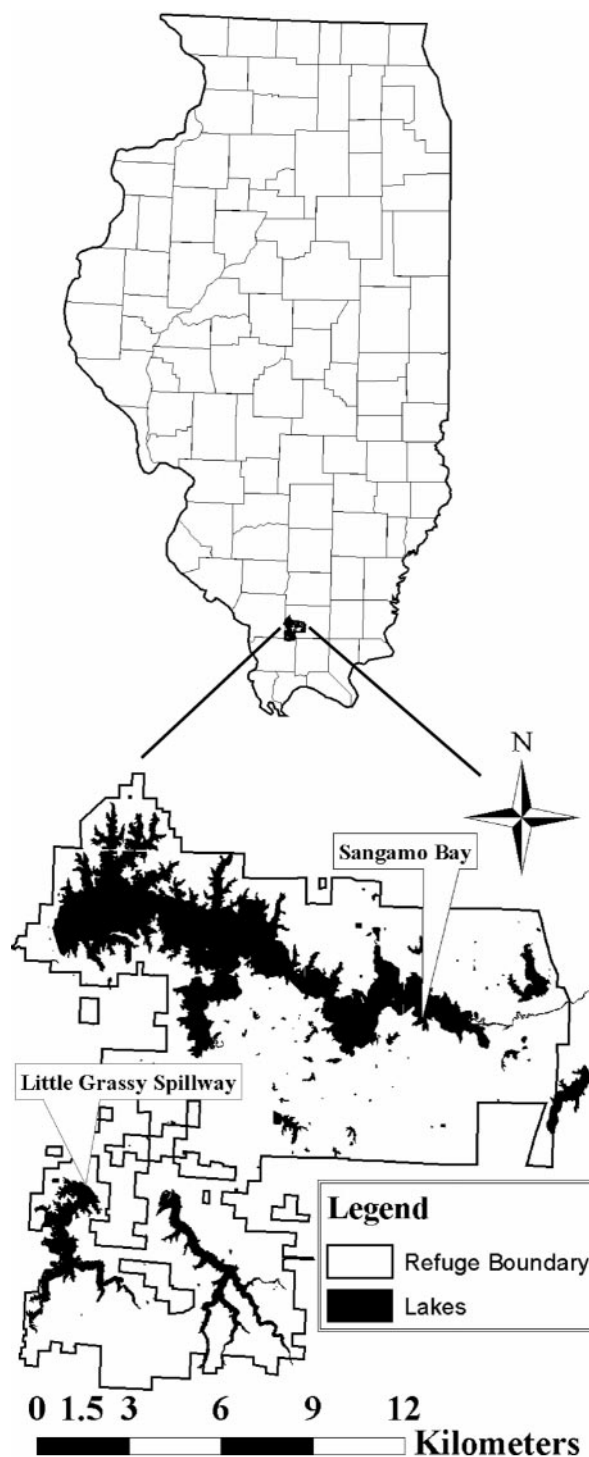


Fig. 1. Location of the Sangamo Bay area of Crab Orchard Lake and Little Grassy Lake on the Crab Orchard National Wildlife Refuge (CONWR), Williamson County, Illinois, USA.

(Formicidae), moths (Lepidoptera), grasshoppers (Acrididae), and true bugs (Miridae and Pentatomidae). A final group, aquatic beetles (Dytiscidae and Hydrophilidae), were examined individually, because unlike the other insects examined, adults are aquatic and pupae terrestrial. Thus, they potentially have a very different PCB-exposure scenario. Furthermore, they can disperse great distances, and they were not necessarily emerging from our sites.

### *Tree swallow egg and nestling collection*

Eggs and nestlings were selected randomly from 10 nest boxes constructed at the Sangamo Bay study area and from 10 nest boxes at the Little Grassy study area. Egg samples were collected 1 to 2 d after being laid, and nestling samples were collected at approximately 15 d of age. Eleven samples of three eggs per sample and 10 nestlings were collected at the Sangamo Bay study area. Four samples of three eggs per sample and four nestlings were collected at the Little Grassy study area. Nestlings were euthanized in the field by cervical dislocation and transported to the Crab Orchard National Wildlife Refuge Headquarters, Marion, Illinois, USA, where the gut contents were removed by dissection and placed into sample jars. Gut contents were stored at  $-20^{\circ}\text{C}$  before content and contaminant analysis. The gastrointestinal tract and carcass were placed in a sample jar and stored at  $-20^{\circ}\text{C}$  or on dry ice before contaminant analysis. Immediately before PCB extraction, dominant insects within tree swallow guts were identified. For this procedure, the samples were scanned under a dissecting microscope for intact specimens, which were then identified to the lowest possible taxonomic level (usually family or genus) and ranked according to their relative abundance in the sample.

### *PCB analyses*

Polychlorinated biphenyl concentrations were measured in sediment, insects, and tree swallow gut contents at Southern Illinois University, Carbondale, Illinois, USA, using modified U.S. Environmental Protection Agency (U.S. EPA) SW-846 methods for organic analytes [16]. All samples were extracted within 14 d of collection and analyzed within 60 d after extraction. Sediment extraction was performed using a sonication technique (U.S. EPA Method 3550B [16]). The extraction solvent consisted of 5:1 (v:v) acetone:hexane for the first extraction, followed by 1:1 (v:v) acetone:hexane solution and 1:5 (v:v) acetone:hexane solution for the second two extractions. Solvent was added at a 3:1 solvent to sediment ratio. Following extraction, the sample was dried with anhydrous  $\text{Na}_2\text{SO}_4$  and filtered. The extract was evaporated to a final volume of 5 ml. The concentrated extract was cleaned using sulfuric acid to remove organic interferences, U.S. EPA Method 3665A [16] and copper powder to remove sulfur contamination, and U.S. EPA Method 3660B [16]. Samples were stored at  $4^{\circ}\text{C}$  until further analysis.

For insect and tree swallow gut content extractions, the weighed sample was placed in a mortar and pestle and then ground until macerated. The tissue was removed to a clean screw-cap vial. The mortar and pestle was rinsed with 10 ml of 1:1 (v:v) methylene chloride:acetone, and the rinse solvent also was added to the vial. This was repeated twice with 5 ml of 1:1 (v:v) methylene chloride:acetone. Samples were then placed in an ultrasonic bath (Model 3510R-MTH; Branson, Danbury, CT, USA) for 20 min, after which the solvent was removed and filtered. Next, 10 ml of solvent were added to the tissue in the vial, and the extraction step was repeated. The resulting extract was evaporated, cleaned, and stored as reported for sediment extracts.

Samples were analyzed for 32 specific individual PCB congeners selected for analysis based on a combination of factors, including presence in Aroclor 1254, previous detection in lake sediment in 1999 to 2000 [17], and previous detection in starling nestlings in 1995 and 1996 [14]. Analysis was conducted using an Agilent Technologies (Palo Alto, CA, USA) 6890

Series gas chromatograph system equipped with an electron-capture detector following protocols from U.S. EPA Method 8082 [16]. Quality assurance/quality control included dual-column confirmation (DB-608 and DB-5; J&W Scientific, Folsom, CA, USA), an extraction blank, either a matrix spike or blank spike, and a duplicate for each extraction batch. The lowest value between the two columns was reported for each analyte. In addition, two surrogates (4,4'-dibromooctofluorobiphenyl and decachlorobiphenyl) were added to samples before the addition of solvent. Each daily run or sequence included a solvent blank and at least four calibration standards. A calibration verification standard was run every 10 samples to ensure that the calibration curve was within 15% of the calibration range. Extraction efficiencies for all media and analytes ranged from 60 to 129% for all congeners, with recoveries between 75 and 95% for the 15 congeners reported here. Relative percentage differences between duplicate samples ranged from 0.7 to 13%. Reporting limits for the method were  $1.0\ \mu\text{g}/\text{kg}$  in sediment,  $2.0\ \mu\text{g}/\text{kg}$  in insects and gut content, and from 0.1 to 1.0 for egg and nestling data.

Polychlorinated biphenyl concentrations were measured in tree swallow eggs and nestlings by B&B Laboratories (College Station, TX, USA). Extraction methods followed U.S. EPA Method 3545, pressurized fluid extraction [16]. Resulting extracts were evaporated and cleaned as reported for sediment and then stored at  $4^{\circ}\text{C}$  until analysis. Tree swallow egg and nestling samples were analyzed using U.S. EPA Method 8082 [16] as described previously.

Of the 32 congeners included in the original analysis, 15 were at levels greater than the reporting limits and generally had limited matrix interferences, as suggested by good confirmation between columns. Thus, the present study focuses on the 15 PCB congeners: 49, 52, 66, 87, 99, 101, 105, 110, 118, 128, 138, 153/132, 156, 170, and 180. These congeners represent 55% of Aroclor 1254 and 38% of Aroclor 1260 [18]. Total congener sum values (i.e.,  $\Sigma\text{PCB}$ ) were calculated by summing the concentration of these 15 congeners. Toxic equivalents were determined for dioxin-like congeners (PCBs 105, 118, and 156) by multiplying concentration by toxic equivalency factors reported for birds (0.0001, 0.00001, and 0.0001, respectively) [19]. Total TEQs ( $\Sigma\text{TEQ}$ ) were calculated by summing TEQ values determined for PCB congeners 105, 118, and 156.

### *TOC and tissue lipid determinations*

All sediment samples were analyzed for TOC. The TOC analysis was conducted at the Great Lakes Environmental Research Laboratory, Ann Arbor, Michigan, USA, and determined for two subsamples as reported previously [20].

Tissue lipid for caddisflies and male and female *Chironomus* spp. was determined spectrophotometrically as described previously [21]. Tissue lipid for tree swallow egg and nestling tissue samples was determined via gravimetric measurement using a 100-ml aliquot of the sample extract before cleanup.

### *Data analysis*

Sediment and tree swallow nestling  $\Sigma\text{PCB}$  concentrations were  $\log(x + 1)$  transformed and compared between the contaminated and reference sites with analysis of variance (ANOVA) [22]. The BSAFs were calculated as the ratio of lipid-normalized PCB ( $\mu\text{g}/\text{kg}$ ) in the biota to TOC-normalized PCB ( $\mu\text{g}/\text{kg}$ ) in the sediment. Between hypothesized trophic levels, BMFs were calculated as the ratio of tree swallow nestlings

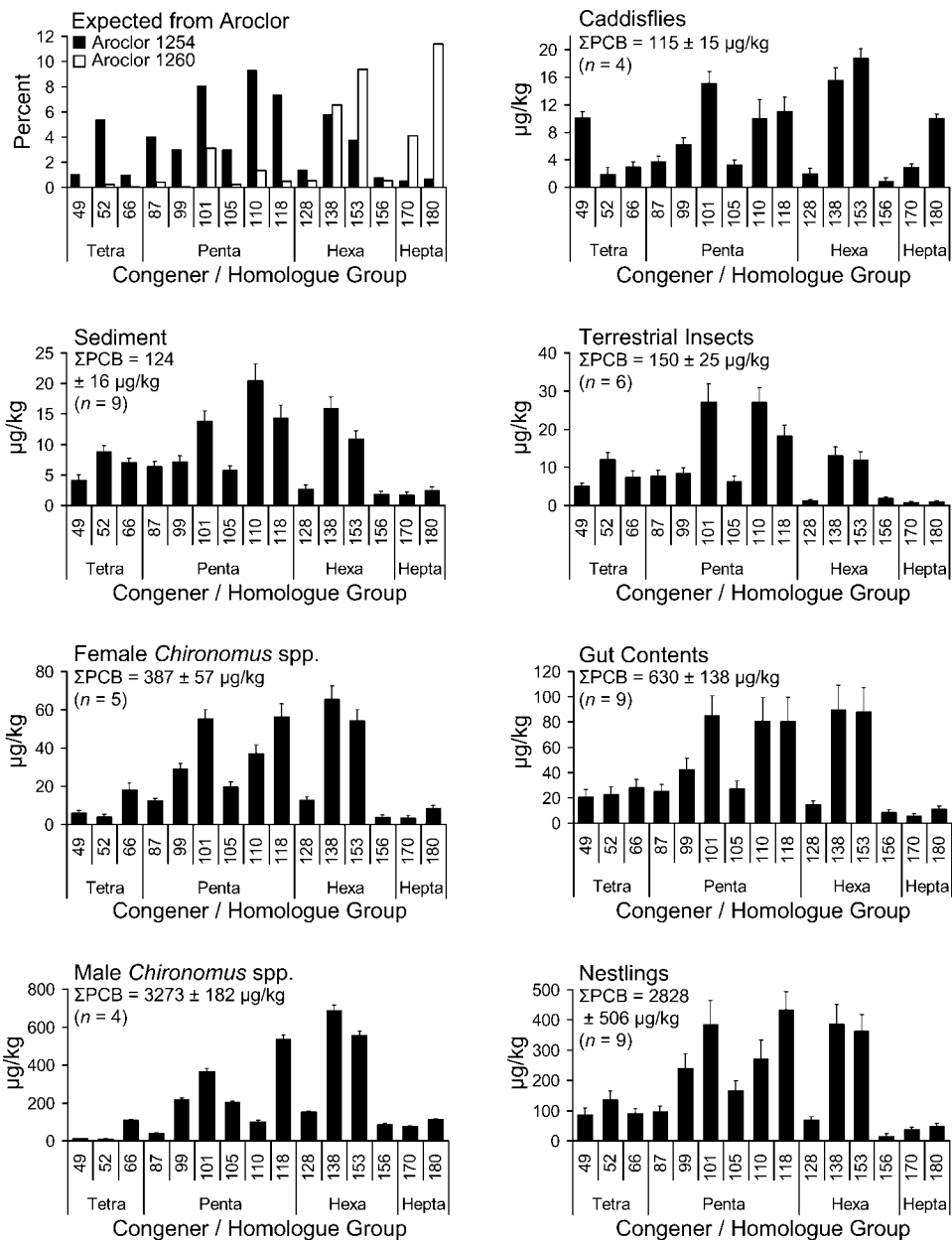


Fig. 2. Mean ( $\pm$  standard error) congener and total polychlorinated biphenyl ( $\Sigma$ PCB) concentrations for sediment, emergent aquatic insect taxa, terrestrial insect taxa, tree swallow (*Tachycineta bicolor*) nestlings, and their gut contents at Sangamo Bay, Crab Orchard National Wildlife Refuge, Williamson County, Illinois, USA, in 2004. Also shown is the percentage of each congener expected from Aroclors 1254 (solid bars) and 1260 (open bars) from Frame et al. [18]. Scale on the y-axis varies among matrices to show the perspective of relative congener pattern.

( $\mu\text{g}/\text{kg}$  wet wt) to insect or tree swallow gut contents ( $\mu\text{g}/\text{kg}$  wet wt). Concentrations less than the reporting limit were treated as zero for the purposes of calculations and statistical analyses.

Variation in concentration-based congener patterns among sediment, aquatic and terrestrial insects, and tree swallow gut contents was examined with principal component analysis (PCA) using a centered variance-covariance cross-products matrix with the PC-ORD software [23]. Before analysis, congener sum concentration data were  $\log(x + 1)$  transformed. Analysis of variance was used to compare sample scores on the first two axes for sediment, gut content, terrestrial insects, and aquatic insect groups that had more than one sample. Additional principal components considered for the ANOVA were those having eigenvalues greater than the eigenvalues

calculated using a broken-stick model [24]. For a significant ANOVA, a Student-Newman-Keuls multiple-comparison test was used to compare means.

## RESULTS

### Total PCB concentrations

Total PCB concentrations in sediment ( $123.65 \pm 15.93 \mu\text{g}/\text{kg}$ ) and tree swallow nestlings ( $2,827.76 \pm 505.67 \mu\text{g}/\text{kg}$ ) were greater at Crab Orchard Lake than at the reference site at Little Grassy Lake (sediment,  $11.95 \pm 1.03 \mu\text{g}/\text{kg}$ ; nestlings,  $32.66 \pm 4.11 \mu\text{g}/\text{kg}$ ;  $p < 0.001$ ). Polychlorinated biphenyl concentrations generally increased with each trophic level, from sediment to aquatic emergent and terrestrial insects to nestlings at Crab Orchard Lake (Fig. 2). The mean gut content  $\Sigma$ PCB



Table 1. Mean ( $\pm$  standard error) sediment concentration ( $n = 9$ ) and biota-sediment accumulation factors (BSAFs) for individual polychlorinated biphenyl (PCB) congeners in female *Chironomus* spp., male *Chironomus* spp., and tree swallow (*Tachycineta bicolor*) nestlings at Sangamo Bay, Crab Orchard National Wildlife Refuge, Williamson County, Illinois, USA<sup>a</sup>

Homologue group	PCB congener	Sediment ( $\mu\text{g}/\text{kg}$ )	<i>Chironomus</i> spp.		Tree swallow nestlings
			Female	Male	
Tetra	49	3.25 $\pm$ 0.75	0.44	1.32	2.38
	52	6.70 $\pm$ 1.07	0.14	0.50	3.78
	66	5.38 $\pm$ 0.82	0.81	7.37	2.56
Penta	87	4.99 $\pm$ 0.94	0.59	2.87	2.68
	99	5.58 $\pm$ 1.10	1.24	14.05	6.71
	101	10.70 $\pm$ 1.99	1.22	12.25	10.68
	105	4.49 $\pm$ 0.88	1.05	16.25	4.68
	110	15.74 $\pm$ 3.05	0.56	2.29	7.45
Hexa	118	11.23 $\pm$ 2.30	1.19	17.12	12.24
	128	2.32 $\pm$ 0.81	1.33	23.75	1.91
	138	12.35 $\pm$ 2.34	1.26	19.93	10.83
Hepta	153 <sup>b</sup>	8.52 $\pm$ 1.67	1.52	23.45	10.37
	156	1.58 $\pm$ 0.51	0.56	19.78	0.36
	170	1.45 $\pm$ 0.45	0.58	19.10	1.08
	180	2.01 $\pm$ 0.60	1.00	20.62	1.41
	$\Sigma\text{PCB}^c$	96.27 $\pm$ 18.84	0.95	12.19	2.67
	$\Sigma\text{TEQ}^d$	0.72 $\pm$ 0.16	0.97	17.16	2.83

<sup>a</sup> The BSAFs for each PCB congener and toxic equivalents (TEQs) were calculated as the ratio of concentration in biota (wet-wt basis) to sediment (dry-wt basis). Sediment and biota concentrations were normalized to the percentage of total organic carbon and percentage lipid, respectively.

<sup>b</sup> Represents a congener 153/132 coelution.

<sup>c</sup>  $\Sigma\text{PCB}$  = sum of PCB concentrations.

<sup>d</sup>  $\Sigma\text{TEQ}$  = sum of toxic equivalency factors (TEFs)  $\times$  concentration values for dioxin-like congeners (TEFs: PCB 105 = 0.0001, PCB 118 = 0.00001, and PCB 156 = 0.0001) [19].

concentration of 630  $\mu\text{g}/\text{kg}$  (Fig. 2) was 73% of the mean  $\Sigma\text{PCB}$  concentration for all insect samples (862  $\mu\text{g}/\text{kg}$ ).

Among field-collected insect samples, male *Chironomus* spp. had a roughly 8.5-fold higher  $\Sigma\text{PCB}$  concentration than females (Fig. 2). When lipid normalized, this difference was even greater (i.e., 12.8-fold higher in males), with male and female lipid-normalized  $\Sigma\text{PCB}$  concentrations of 1,173  $\pm$  65 and 91  $\pm$  14  $\mu\text{g}/\text{kg}$ , respectively. Female *Chironomus* spp. had greater percentage moisture (females, 60%; males, 57%), individual dry weight (females, 3.15 mg/individual; males, 1.07 mg/individual), and percentage lipid (females, 4.21%; males, 2.79% lipid) than male *Chironomus* spp.

Caddisflies and terrestrial insects had the lowest  $\Sigma\text{PCB}$  concentrations of all insect groups tested (Fig. 2). Caddisflies were abundant at Crab Orchard Lake, but all belonged to the stream-dwelling family Hydropsychidae, indicating that they had immigrated from a nearby lotic system. Furthermore, PCB congener 180 was high in caddisflies relative to other insect taxa (Fig. 2), supporting the idea that they had immigrated from a lotic site that contained a source of different and higher-chlorinated PCBs (i.e., Aroclor 1260). Total PCB concentrations for Chironomidae and aquatic beetles (Dytiscidae) were 581.4 and 1,766  $\mu\text{g}/\text{kg}$ , respectively, and were from single replicates because of the low biomass collected for these groups.

In addition to diet, PCB sources to nestlings also may include deposition in eggs that females accumulated at migratory and over-wintering habitats with unknown PCB concentrations and patterns. This source must be quantified when examining trophic transfer processes and evaluating site-derived contaminant impacts [25]. It was estimated that the egg accounted for approximately 6% of the  $\Sigma\text{PCB}$  concentration in nestlings at Sangamo Bay (calculated as mean total PCB mass per egg divided by mean total PCB mass per nestling). Thus, approximately 94% of PCB accumulation observed in nestlings oc-

curred through dietary uptake and can be attributed to trophic transfer processes.

#### Bioaccumulation and biomagnification

The BSAFs for tree swallow nestlings and female and male *Chironomus* spp. were calculated for each congener,  $\Sigma\text{PCB}$ , and  $\Sigma\text{TEQ}$  (Table 1). Male *Chironomus* spp. had a higher BSAF (12.19) compared to female *Chironomus* spp. (0.95), indicating that they had greater accumulation of PCBs from the sediment (Table 1). The BSAF for tree swallow nestlings based on  $\Sigma\text{PCB}$  was 2.67. The BSAFs for  $\Sigma\text{TEQ}$  were similar to those found for  $\Sigma\text{PCB}$  within each biota (Table 1). Among individual congeners, BSAFs varied greatly within each biotic group (e.g., 0.36–12.24 among congeners in tree swallow nestlings). Of particular interest is that the dioxin-like PCB congener 118 had the greatest BSAF in nestlings, whereas another congener, PCB 156, had the lowest BSAF among all congeners examined in the present study (Table 1). In terms of chlorine homolog groups, penta- and hexachlorinated congeners generally had greater enrichment in nestlings and female *Chironomus* spp. compared with tetra- and heptachlorinated congeners (Table 1). For male *Chironomus* spp., greatest enrichment generally occurred for hexa- and heptachlorinated congeners (Table 1).

The BMFs for tree swallow nestlings varied among PCB congeners within each potential diet source (i.e., tree swallow gut contents, female and male *Chironomus* spp., caddisflies, and terrestrial insects) (Table 2). The nestling BMF for  $\Sigma\text{PCB}$  concentrations calculated from female *Chironomus* spp. was most similar to that found for gut contents as compared to all other insect groups examined (Table 2). The calculated nestling BMF for  $\Sigma\text{PCB}$  concentrations for the single Chironomidae sample (not shown in Table 2) was 4.86, similar to the value of 4.49 determined for gut contents. Nestling BMFs relative

Table 2. Mean ( $\pm$  standard error) tree swallow (*Tachycineta bicolor*) nestling concentration ( $n = 9$ ) and biomagnification factors (BMFs) of individual polychlorinated biphenyl (PCB) congeners, total PCB concentration ( $\Sigma$  PCB), and toxic equivalents (TEQs) from gut content and insect samples at Sangamo Bay, Crab Orchard National Wildlife Refuge, Williamson County, Illinois, USA<sup>a</sup>

PCB congener	Nestlings ( $\mu\text{g}/\text{kg}$ )	Gut contents	<i>Chironomus</i> spp.			Terrestrial insects
			Female	Male	Caddisflies	
49	87.07 $\pm$ 21.73	4.23	14.59	7.26	8.57	17.15
52	135.71 $\pm$ 30.05	6.03	34.08	14.44	70.75	11.29
66	90.81 $\pm$ 16.33	3.23	4.96	0.82	30.45	12.06
87	96.43 $\pm$ 19.75	3.84	7.83	2.41	25.75	12.36
99	240.38 $\pm$ 47.33	5.64	8.26	1.10	38.77	28.12
101	384.10 $\pm$ 80.17	4.52	6.97	1.05	25.36	14.13
105	167.41 $\pm$ 31.23	6.19	8.44	0.82	52.03	26.24
110	270.33 $\pm$ 62.20	3.34	7.25	2.69	27.02	10.01
118	434.40 $\pm$ 59.87	5.40	7.71	0.81	39.33	23.78
128	68.20 $\pm$ 11.98	4.73	5.27	0.44	33.97	53.70
138	385.82 $\pm$ 65.47	4.31	5.89	0.56	24.81	29.47
53 <sup>b</sup>	364.39 $\pm$ 54.54	4.12	6.70	0.65	19.34	30.66
156	14.30 $\pm$ 9.24	1.68	3.82	0.16	17.18	7.61
170	38.61 $\pm$ 6.32	6.76	10.96	0.50	13.27	49.86
180	49.79 $\pm$ 7.71	4.47	5.87	0.43	4.96	48.86
$\Sigma$ PCB	2,827.76 $\pm$ 505.67	4.49	7.31	0.86	24.68	18.88
$\Sigma$ TEQ <sup>c</sup>	22.52 $\pm$ 4.32	5.17	7.71	0.65	43.68	22.32

<sup>a</sup> Nestling BMFs were calculated as the ratio of concentration in tree swallow nestlings to gut contents and insects. All concentrations and BMFs are reported on a wet-weight basis.

<sup>b</sup> Represents a congener 153/132 coelution.

<sup>c</sup>  $\Sigma$ TEQ = sum of toxic equivalency factors (TEFs)  $\times$  concentration values for dioxin-like congeners (TEFs: PCB 105 = 0.0001, PCB 118 = 0.00001, and PCB 156 = 0.0001) [19].

to caddisflies and terrestrial insects suggest lower concentrations for almost all PCB congeners compared to female and male *Chironomus* spp. and gut contents (Fig. 2).

#### PCB congener pattern analysis

Polychlorinated biphenyl congener patterns for most sample types, including sediment, were similar to an Aroclor 1254 pattern, with some deviation that may suggest environmental degradation and/or biotransformation (Fig. 2). The Aroclor 1254 pattern is consistent with the known contaminant source at the site [14]. For some samples, such as caddisflies, chromatographic analyses suggested a possible combination of two Aroclors (i.e., 1254 and 1260). No other Aroclors were detected in the samples.

The ordination analysis of sediment, insect, and tree swallow gut content samples in PCB congener space suggested variation in pattern among sample types (Fig. 3). The cumulative variance explained by the first two axes of the PCA was 89.2%, with 80.7% attributed to the first axis and 8.5% to the second axis. Most of the variation was explained on the first two axes, with the remaining axes having eigenvalues well below those calculated using a broken-stick model [24]. Congeners with the greatest negative factor loadings on the first axis were PCBs 128, 138, 153/132, and 156, whereas PCB congeners 49, 52, 87, and 110 had the greatest factor loadings in the positive direction on the first axis (Fig. 3). On the second axis, PCB congeners 128, 153/132, 170, and 180 had the greatest positive factor loadings, and PCB congeners 49, 52, 87, and 110 had the greatest negative factor loadings (Fig. 3). The dioxin-like PCB congeners 105 and 118 had negative factor loadings on the first axis similar to those listed above and shown in Figure 3.

Sample scores varied among groups on both PCA axes ( $p < 0.0001$ ). One interpretation of the ordination diagram is that two samples with very similar concentration-based congener

patterns plot nearest to each other within the ordination diagram. Thus, separation was apparent between terrestrial-based and aquatic-based diet items (Fig. 3). Mean sample scores for male and female *Chironomus* spp. and gut contents were lower than those for terrestrial-based taxa, caddisflies, and sediment on the first axis ( $p < 0.05$ ). Also, on the first axis, scores for female *Chironomus* spp. were similar to those for gut contents

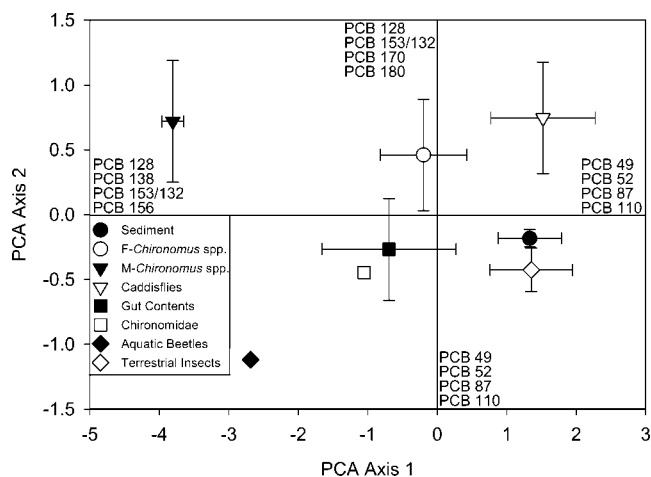


Fig. 3. Principal component analysis (PCA) of sediment, insect, and tree swallow (*Tachycineta bicolor*) nestling gut content samples in polychlorinated biphenyl (PCB) congener ordination space from Sangamo Bay, Crab Orchard National Wildlife Refuge, Williamson County, Illinois, USA, in 2004. Symbols are mean component scores for sediment, aquatic emergent insects (male and female *Chironomus* spp. and caddisflies), terrestrial insects, and tree swallow gut content samples. Error bars are  $\pm 2$  standard errors, and sample sizes can be found in Figure 2. Symbols indicating aquatic beetles and Chironomidae are scores for single samples. Polychlorinated biphenyl congeners labeled on the ends of each axis are those with high factor loadings for the positive or negative end of the axis. F = female, M = male.

( $p > 0.05$ ). On the second axis, sample scores for all emergent insects (i.e., male and female *Chironomus* spp. and caddisflies) were greater than those for gut contents, sediment, and terrestrial insects ( $p < 0.05$ ). The PCB congener pattern observed for terrestrial insects was similar to that observed for sediment samples ( $p > 0.05$ ) (Fig. 3). The PCB congener pattern in male *Chironomus* spp., based on scores from both components, varied the most from all other sample types.

## DISCUSSION

### PCBs and trophic transfer

The present study clearly demonstrates that tree swallow nestlings at Sangamo Bay bioaccumulated PCBs as a result of trophic transfer processes, in which the original source of contamination was sediment. Previous work at Crab Orchard Lake reported PCB bioaccumulation in insectivorous passerines [14], but the specific patterns and processes of trophic transfer were not examined. In the present study, emphasis was placed on the ecological mechanisms of trophic transfer from the contaminant source (sediment) to a higher-trophic-level organism (tree swallow). At Crab Orchard Lake,  $\Sigma$ PCB concentrations generally increased with each trophic level from sediment to insect prey to tree swallow nestlings. Because  $\Sigma$ PCB concentrations in gut content samples were within the range measured for emergent insects and all gut content samples contained chironomids, the sediment to Chironomidae to tree swallow food chain was one likely pathway for trophic transfer of PCBs at this site.

Total PCB concentrations in nestlings during the present study were approximately 10- to 20-fold lower than those reported in other studies of PCB-contaminated sites [2,3,26]. However,  $\Sigma$ PCB concentrations were similar to those reported at sites in the Fox River drainage and Green Bay, Wisconsin, USA, where ethoxyresorufin-*O*-dealkylase activities of nestling and newly hatched tree swallows were elevated relative to control sites [1]. It must be noted, however, that comparing  $\Sigma$ PCB concentrations among studies may be influenced by dissimilarity in congener lists and may have limited biological relevance. A better estimate may be TEQs, and those reported by Custer et al. [1] (62 and 172  $\mu\text{g}/\text{kg}$ ) were greater than those in the present study (23  $\mu\text{g}/\text{kg}$ ). Interestingly,  $\Sigma$ PCB concentrations in nestlings in the present study were 10-fold lower compared to a study conducted in 1995 on European starlings at the same site with a very similar congener list [14].

Through separation of the genus *Chironomus* from other Chironomidae and examining individual sexes, a great deal of variation in  $\Sigma$ PCB concentration was observed. Males in the genus *Chironomus* had  $\Sigma$ PCB concentrations more than eight-fold greater than those in females. This contradicts the lack of sex-dependent variation in PCB body residues in *Chironomus riparius* determined in laboratory partial life-cycle experiments [27]. However, only single di-, tetra-, and pentachlorinated biphenyls (i.e., PCB congeners 15, 47, and 126) were examined, none of which was included in the congener list of the present study. Why concentration varied between sexes in the present study was unclear. Differences may be related to sex-dependent variation associated with a wide variety of factors, such as larval microhabitat, bioaccumulation rates, growth rates, metabolism, time spent in the sediment before emergence, patterns in pupation or emergence [5], female lipid (and contaminant) loss because of egg-laying, and female immigration from another location. This finding may have implications for dilution effects in trophic transfer of PCBs to

tree swallows, both because female *Chironomus* spp. were larger than males and because tree swallows have been shown to exhibit prey-size selection [28]. More studies involving both field and laboratory experiments and multiple PCB congeners are needed to elucidate fully the possibility of sex-dependent PCB bioaccumulation in emergent insects.

### PCB bioaccumulation and biomagnification

Overall, significant bioaccumulation of  $\Sigma$ PCB in tree swallow nestlings was found relative to sediment concentrations, as indicated by a BSAF of 2.67. This is lower than the BSAF of 9.3 reported previously for the  $\Sigma$ PCB in nestlings [2]. The nestling BSAF value for  $\Sigma$ TEQ in the present study was slightly higher than that for  $\Sigma$ PCB, which supports the hypothesis that dioxin-like congeners may exhibit differential bioaccumulation relative to other congeners [29,30]. However, for the three mono-*ortho*-substituted PCB congeners examined here (i.e., PCB congeners 105, 118, and 156), BSAF values ranged from the highest to the lowest among all congeners. The  $\Sigma$ TEQ BSAF of 2.83 in the present study was more than double that reported by Froese et al. [2] and can be attributed to the bioaccumulation patterns of PCB congener 118.

Bioaccumulation of dioxin-like congeners also may be organism-dependent, with high bioaccumulation rates of non-*ortho*-substituted congeners noted in phytoplankton, zooplankton, *Mysis* spp., *Diporeia* spp. [30], and Atlantic salmon [31]. For emergent aquatic insects in another study [2], a BSAF of 0.3 was reported for non- and mono-*ortho*-substituted PCB congeners, suggesting that they had significantly reduced bioaccumulation of dioxin-like congeners relative to other congeners. This pattern did not correspond with BSAF patterns for female *Chironomus* spp. examined here, in which BSAFs based on  $\Sigma$ PCB concentrations and  $\Sigma$ TEQ values were similar but did not concur with other studies demonstrating that *ortho*-substitution did not affect bioaccumulation of PCB congeners in plankton and fish [32] and in four-horned sculpin liver tissue [33].

Nestling BMFs for  $\Sigma$ PCB in gut contents, female *Chironomus* spp., and other Chironomidae ranged from 4.49 to 7.31, suggesting that female *Chironomus* spp. and other Chironomidae were similar in  $\Sigma$ PCB concentration to diet items consumed by nestlings. These taxa may be important for contaminant contribution to the diet. However, gut contents also could be a mixture of high-PCB-concentration aquatic and terrestrial insects. The BMF from gut contents to nestlings reported here was similar to the BMFs of 3.48 and 6.18 determined for PCB-contaminated sites at Fox River and Green Bay [1]. Also of interest is that the variation in nestling BMFs among congeners was lower for gut content samples than for individual insect groups, suggesting that PCB concentrations in nestlings on an individual congener basis are better represented by gut content samples than by field-collected insects. This also suggests that gut contents may represent an averaging of congener patterns found among a variety of diet items.

### PCB congener pattern analysis

Polychlorinated biphenyl congener patterns for tree swallow nestlings in the present study were similar to those observed in 15-d-old European starling (*Sturnus vulgaris*) nestlings at Crab Orchard Lake almost a decade earlier [14]. Concentrations of 13 congeners common to both studies, including



those that may be dioxin-like (e.g., PCBs 105 and 118), were within 3.6% between the two species on a percentage basis.

The ordination analysis indicated that a PCB congener patterns of tree swallow gut contents are variable and cannot be attributed entirely to a particular insect taxon in the diet. Both because tree swallows at Crab Orchard Lake exhibited dietary plasticity and because diet items varied in their PCB congener patterns, some gut content samples had PCB congener patterns similar to that observed for terrestrial-based diet items, whereas others reflected PCB congener patterns of emergent aquatic insects. This can be demonstrated further by examining the concentration ratio of PCB congeners 101 and 138 among insects and gut contents. The ratio ranged from 0.74 to 0.96 in female *Chironomus* spp., from 0.52 to 0.55 in male *Chironomus* spp., and from 1.32 to 8.07 in terrestrial insects. In gut content samples, the ratio of PCB 101 to PCB 138 ranged from 0.64 to 2.75, overlapping those of the three insect taxa mentioned above and further suggesting a variety of diet items in tree swallow gut contents. Similarly, Russell et al. [10] showed that a large proportion of terrestrial insects in the diet of a fish species shifted PCB congener patterns relative to other fish species that fed primarily on aquatic-based diet items within the same river habitat. Variation in PCB congener patterns also was observed among aquatic-based diet items, such as mayfly and caddisfly larvae and zooplankton [10], similar to the congener pattern variability among aquatic insect diet items in the present study.

These patterns demonstrate the complexity of trophic pathways of contaminants from sediment to some higher-trophic-level organisms and have direct implications to studies that may use only measured contaminant concentrations in sediment or prey taxa (e.g., emergent insects) to estimate risk to higher trophic levels. Furthermore, examining only a segment of the tree swallow diet may significantly under- or overestimate bioaccumulation of specific PCB congeners to this species. For example, some of the separation of sample points on the first and second axes of the PCA was described by the concentration of PCB congener 52; it was relatively high in the terrestrial taxa samples compared to other congeners. As such, tree swallow nestling BMFs calculated from female *Chironomus* spp. were very large relative to the BMF for the gut content samples that consisted of a composite of diet items that included terrestrial insects, suggesting that terrestrial diet items were a significant source of this particular congener to nestlings. This idea is supported further by the similarity (difference, <1%) in the relative abundance of PCB 52 in nestling European starlings, a species in which nestlings were likely also fed terrestrial-derived insects, measured at the same study site [14]. In addition, in recently investigated tree swallow stomach contents that had the highest reported concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in avian diets, 37% of the diet items were terrestrial insects [34]. In that study, however, the contaminant contribution that could be attributed to each of the aquatic and terrestrial diet items was unclear.

Tree swallow dietary plasticity, as supported by breadth of identified gut contents in the present study, also may have implications for the reported temporal variation in contaminant trophic transfer and accumulation rates among successive tree swallow clutches at a site both during a single breeding season [1] and among years [26]. This may be explained by variation in PCB congener patterns and  $\Sigma$ PCB concentration among potential tree swallow prey items demonstrated in the present study in conjunction with seasonal shifts in the abundance of

insect prey taxa. Tree swallow foraging and diet composition often are dependent on abundance of prey items at a site [8]. Thus, as individual taxa become more abundant at a contaminated site, their percentage composition in the diet would increase and, ultimately, influence PCB accumulation in tree swallows.

## CONCLUSION

Results from the present study indicate that bioaccumulation of PCBs occurred in tree swallow nestlings at Crab Orchard Lake. By examining PCB concentrations in both potential insect diet items as well as nestling gut contents, the present study documented that some insect groups contributed proportionately more PCBs to nestlings on a  $\Sigma$ PCB basis compared with other groups (i.e., based on nestling BMFs relative to each insect group). However, when examining concentration-based congener patterns in gut contents, contribution to gut contents may have occurred from a wide variety of insect taxa. Some gut content samples had very similar congener patterns to terrestrial insects, whereas others were more similar to emergent aquatic insects. This pattern was supported further by both the observed variation of  $\Sigma$ PCB concentrations among gut content samples (coefficient of variation, 65.5%) and the qualitative diet analysis, indicating variation of diet items within and among gut content samples. Thus, modeling trophic linkages for contaminant transfer to insectivorous birds using only one segment of available diet resources (e.g., solely emergent aquatic insects), neglecting protandrous (i.e., sex-skewed) emergence patterns, or not considering diel patterns in emergence may influence or bias calculation of PCB BMFs in insectivorous birds. Because of the large variation in nestling PCB concentrations observed in the present study and at other contaminated sites [2,3], we suggest that future research consider the complexity of food webs when examining PCB accumulation in higher-trophic-level organisms, such as insectivorous birds. Furthermore, this variation in PCB residues in nestlings and gut contents may be explained best using quantitative trophic source identification techniques [35].

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