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DNA metabarcoding reveals provisioning of pollution-sensitive aquatic insects, resource partitioning, and dietary shifts among breeding Neotropical migratory songbirds in a riparian habitat

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DNA METABARCODING REVEALS PROVISIONING OF POLLUTION-SENSITIVE AQUATIC INSECTS, RESOURCE PARTITIONING, AND DIETARY SHIFTS AMONG BREEDING NEOTROPICAL MIGRATORY SONGBIRDS IN A RIPARIAN HABITAT

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Brian K. Trevelline

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ABSTRACT

DNA METABARCODING REVEALS PROVISIONING OF POLLUTION-SENSITIVE AQUATIC INSECTS, RESOURCE PARTITIONING, AND DIETARY SHIFTS AMONG BREEDING NEOTROPICAL MIGRATORY SONGBIRDS IN A RIPARIAN HABITAT

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Elucidating the diet of Neotropical migratory birds is essential to our understanding of their ecology and to their long-term conservation. Beyond broad taxonomic or morphological categories, however, the diet of Neotropical migrants is poorly documented. Using the molecular techniques of DNA barcoding and next-generation sequencing, we elucidated the diet of Neotropical migratory songbirds breeding in the riparian zones of headwater Appalachian streams. This approach resulted in a genus- or species-level description of diets that improved the current understanding of how songbirds utilize aquatic prey resources in riparian habitats. Furthermore, our approach revealed that breeding songbirds partition prey resources within a shared riparian habitat. Despite substantial differences in foraging strategy, we provide evidence that syntopic riparian species opportunistically prey upon pollution-sensitive emergent aquatic insects, thus emphasizing the importance of aquatic resource subsidies for songbirds breeding in riparian habitats. For the stream-dependent Louisiana Waterthrush, the provisioning of aquatic insects was significantly higher than other riparian songbirds. As a result, waterthrush breeding in riparian habitats with reduced availability of aquatic arthropods expanded their diet by targeting a more diverse array of insects that included significantly more terrestrial taxa. In addition to providing support for our hypothesis that Louisiana Waterthrush compensate for food shortages by targeting terrestrial arthropods in degraded riparian habitats, our findings emphasize the vulnerability of Louisiana Waterthrush to anthropogenic disturbances that compromise stream quality and the availability of pollution-sensitive aquatic insects.

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CONTRIBUTIONS

Chapter 1: Brian K. Trevelline, Steven C. Latta, Tim Nuttle, and Brady A. Porter conceived the idea, design, and experiment (supervised research, formulated question or hypothesis); B.K.T. and Leesia C. Marshall performed the experiments (collected data, conducted the research); B.K.T. wrote the paper with edits from S.C.L., L.C.M., T.N., and B.A.P.; B.K.T. developed and designed the methods; B.K.T. analyzed the data; and S.C.L, T.N., and B.A.P. contributed substantial materials, resources, and funding.

Chapter 2: B.K.T., T.N., B.A.P., and S.C.L. collectively conceived and designed this study as an extension of S.C.L.'s long-term research on Louisiana Waterthrush. B.K.T. and Brandon D. Hoenig conducted the fieldwork (with guidance from T.N., B.A.P., and S.C.L.); B.K.T. developed the field and laboratory protocols, conducted molecular work (with B.D.H.), performed statistical analyses (with Nathan L. Brouwer), and wrote the manuscript in the laboratory of B.A.P.

Chapter 3: B.K.T., T.N., B.A.P., and S.C.L. collectively conceived and designed this study as an extension of S.C.L.'s long-term research on Louisiana Waterthrush. B.K.T. and B.D.H. conducted the fieldwork (with guidance from T.N., B.A.P., and S.C.L.). Aquatic macroinvertebrates were identified by Marisa Logan. Emergent EPT taxa were identified and enumerated by B.K.T. and Zachary D. Steffensmeier. B.K.T. developed the field and laboratory protocols, conducted molecular work (with B.D.H.), performed statistical analyses (with N.L.B.), and wrote the manuscript in the laboratory of B.A.P.

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CHAPTER ONE

Molecular analysis of nestling diet in a long-distance Neotropical migrant, the Louisiana Waterthrush (*Parkesia motacilla*)

Elucidating the diet of Neotropical migratory birds is essential to our understanding of their ecology and to their long-term conservation. Reductions in prey availability negatively impact Neotropical migrants by affecting their survival as both nestlings and adults. Beyond broad taxonomic or morphological categories, however, the diet of Neotropical migrants is poorly documented. Using the molecular techniques of DNA barcoding and next-generation sequencing, we elucidated the diet of Louisiana Waterthrush (Parkesia motacilla) nestlings in Arkansas and Pennsylvania, USA. Waterthrush have been shown to respond negatively to the reduced availability of aquatic insects in the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT taxa). We hypothesized that Louisiana Waterthrush nestling diet would be primarily composed of these pollution-sensitive aquatic taxa, and that changes in the riparian insect community would be reflected in their diet. Unexpectedly, the orders Lepidoptera (92%) and Diptera (70%) occurred frequently in the diet of Louisiana Waterthrush nestlings. Among EPT taxa, only the order Ephemeroptera (61%) was frequently detected whereas Plecoptera (7%) and Trichoptera (1%) were poorly represented. The frequency at which aquatic Ephemeroptera and terrestrial Lepidoptera were detected in waterthrush nestling diet differed significantly over the nesting period in Pennsylvania but not in Arkansas, suggesting that phenological shifts in the availability of non-EPT prey taxa may be an important yet undescribed factor influencing the foraging ecology of waterthrush on the breeding grounds. Furthermore, these findings suggest that terrestrial insects may be more important to waterthrush nestlings than previously thought, which enhances our understanding of this biological indicator and Neotropical migrant.

1.1 INTRODUCTION

Elucidating the dietary composition and food preferences of migratory birds is essential to understanding their ecology, population dynamics, and conservation. Throughout the annual cycle, the availability of food is considered a major limiting factor for populations of birds that migrate from the Neotropics (Martin 1987; Newton 2004) and has been shown to affect migration departure and return rates (Cooper *et al.* 2015; Studds & Marra 2005), body condition (Latta & Faaborg 2002; Marra *et al.* 1998; Strong & Sherry 2000), breeding and non-breeding distributions (Burke & Nol 1998; Johnson & Sherry 2001), and rates of predation (Hoover *et al.* 1995). Furthermore, food availability has been shown to influence fecundity, which is considered one of the most critical factors for sustaining populations in long-distance Neotropical migrants (Bohning-Gaese *et al.* 1993; Holmes *et al.* 1996; Sherry & Holmes 1992; Sillett & Holmes 2005). Food limitations on the breeding grounds negatively affect fecundity by influencing the survival and body condition of nestlings (Rodenhouse & Holmes 1992; Sillett *et al.* 2000). The influence of food on fecundity is of particular conservation interest given the long-term decline of Neotropical migrants (Robbins *et al.* 1989; Sauer *et al.* 2014; Sauer & Link 2011); therefore, a detailed understanding of diet is essential to identify potential vulnerabilities and develop effective conservation strategies for these important migratory birds.

Currently, our understanding of Neotropical migrant diet is primarily derived from foraging observations and the morphological identification of insect remains from regurgitates (e.g., Robinson & Holmes 1982), gut contents (e.g., Eaton 1958), and fecal material (e.g., Deloria-Sheffield *et al.* 2001). These approaches are labor-intensive, expensive to analyze, require expertise in systematic entomology, and often provide an incomplete understanding of diet due to the limitations associated with identifying digested insect remains (Pompanon *et al.*

2012; Symondson 2002). These limitations are particularly relevant to Neotropical migrants, which commonly prey upon soft-bodied, larval Lepidoptera (e.g., Rodenhouse & Holmes 1992) that may be difficult to identify after digestion (Parrish 1997; Ralph *et al.* 1985). The use of molecular techniques to describe diet from animal feces is an increasingly utilized method for studying trophic interactions. Molecular diet analyses provide ecologists with genus- or species-level taxonomic identification and can be applied to a wide range of study taxa (King *et al.* 2008). Fecal samples are useful for molecular diet studies because they contain residual prey DNA and can be collected with minimal disturbance to the animal (Pompanon *et al.* 2012). DNA barcoding coupled with next-generation sequencing technologies have enabled ecologists to investigate diet using fecal material from felids (Shehzad *et al.* 2012), small mammals (Brown *et al.* 2014), bats (Clare *et al.* 2014), and seabirds (Bowser *et al.* 2013; Deagle *et al.* 2010), all of which would otherwise be difficult to study.

Relative to its widespread use in most major taxonomic groups, however, molecular diet analyses that utilize avian feces are underrepresented in the scientific literature. This deficiency is particularly true of perching birds (order Passeriformes), by far the largest avian order with >50% of all extant avian taxa (Jetz *et al.* 2012). Notably, a recent study of Western Bluebird (*Sialia mexicana*) demonstrated the feasibility of using Illumina sequencing to elucidate diet from fecal samples (Vo & Jedlicka 2014) but has not yet resulted in widespread application. Such molecular approaches enable avian ecologists to generate a comprehensive understanding of diet, which has not been explored in such a descriptive and noninvasive manner.

The Louisiana Waterthrush (*Parkesia motacilla*) is a long-distance Neotropical migratory wood-warbler (family Parulidae). Louisiana Waterthrush are obligate riparian songbirds that occupy linear breeding territories along headwater streams throughout eastern North America

(Mattsson *et al.* 2009; Figure 1.1). Louisiana Waterthrush are considered aquatic insect foraging specialists and an important biological indicator for the integrity of riparian ecosystems (Brooks *et al.* 1998; Mattsson & Cooper 2006; Prosser & Brooks 1998). Waterthrush that nest along degraded streams with suboptimal water quality must establish larger territories to acquire sufficient prey resources (Mulvihill *et al.* 2008), and they lay smaller, delayed clutches (Mulvihill *et al.* 2008) and rarely attempt a second brood (Mulvihill *et al.* 2009). These negative impacts on Louisiana Waterthrush are believed to be the result of reductions in the availability of 3 orders of pollution-sensitive aquatic insects used as biological indicators for stream quality: Ephemeroptera, Plecoptera, and Trichoptera (EPT; Mattsson & Cooper 2006; Mulvihill *et al.* 2008; Wood *et al.* 2016). Previous studies have suggested that EPT taxa are important prey for Louisiana Waterthrush (Mattsson *et al.* 2009) because they were found in the gut contents of 15 individuals in the only published description of waterthrush diet (Eaton 1958). Eaton (1958), however, classified nearly 60% of Louisiana Waterthrush stomach contents as ‘‘undetermined fragments,’’ which, if identified, may have revealed additional important prey items. A detailed description of Louisiana Waterthrush diet is therefore imperative to our understanding of their foraging ecology and has been identified as a priority for future research (Mattsson *et al.* 2009).

In this study, we utilized DNA barcoding and Illumina sequencing to describe the diet of Louisiana Waterthrush nestlings in Arkansas and Pennsylvania, USA. Based on previous diet studies and their documented response to low EPT availability, we hypothesized that Louisiana Waterthrush nestling diet would be predominantly composed of EPT taxa, and that nestling diet would differ over the course of the nesting season by reflecting changes in the riparian insect community.

1.2 MATERIALS AND METHODS

Sample Collection

Louisiana Waterthrush nests were systematically located using behavioral cues along first- and second-order streams in Van Buren and Conway counties, Arkansas (Cedar Creek, Sis Hollow, East Point Remove Creek, and Sunnyside Creek), and Westmoreland County, Pennsylvania (Camp Run, Linn Run, Loyalhanna Creek, and Powdermill Run), beginning in mid-April 2013 (Figure 1.1). Fecal samples were collected by placing nestlings (3–8 days post-hatching) into a clean paper bag for ~ 1 min. Fecal samples were immediately preserved in 20 mL of absolute ethanol and stored at room temperature for a period of ~ 3 months prior to DNA extraction. To investigate potential changes in diet over the course of the nesting period, fecal samples were later subdivided into three 10-day intervals (mid-May = May 12–21; late-May = May 22–31; early-June = June 1–10). Fecal samples collected outside these intervals were not included in analyses that investigated potential changes in diet over the nesting period.

Benthic macroinvertebrates were collected by Surber sampling (Barbour *et al.* 1999) at 10 equidistant riffles along a ~ 2 km segment of each stream that encompassed the foraging territories of all sampled waterthrush nests. All 10 benthic samples were combined to represent the benthic community for the entire reach and repeated every 2 weeks throughout the breeding season. A subsample of 300 (± 20%) individuals (Barbour *et al.* 1999) was randomly selected from each benthic sample, and individuals were morphologically identified to genus by a certified aquatic entomologist (genus-level, Society for Freshwater Science). Relative abundance values were derived based on the number of individuals in an order divided by the total number of individuals in the subsample.

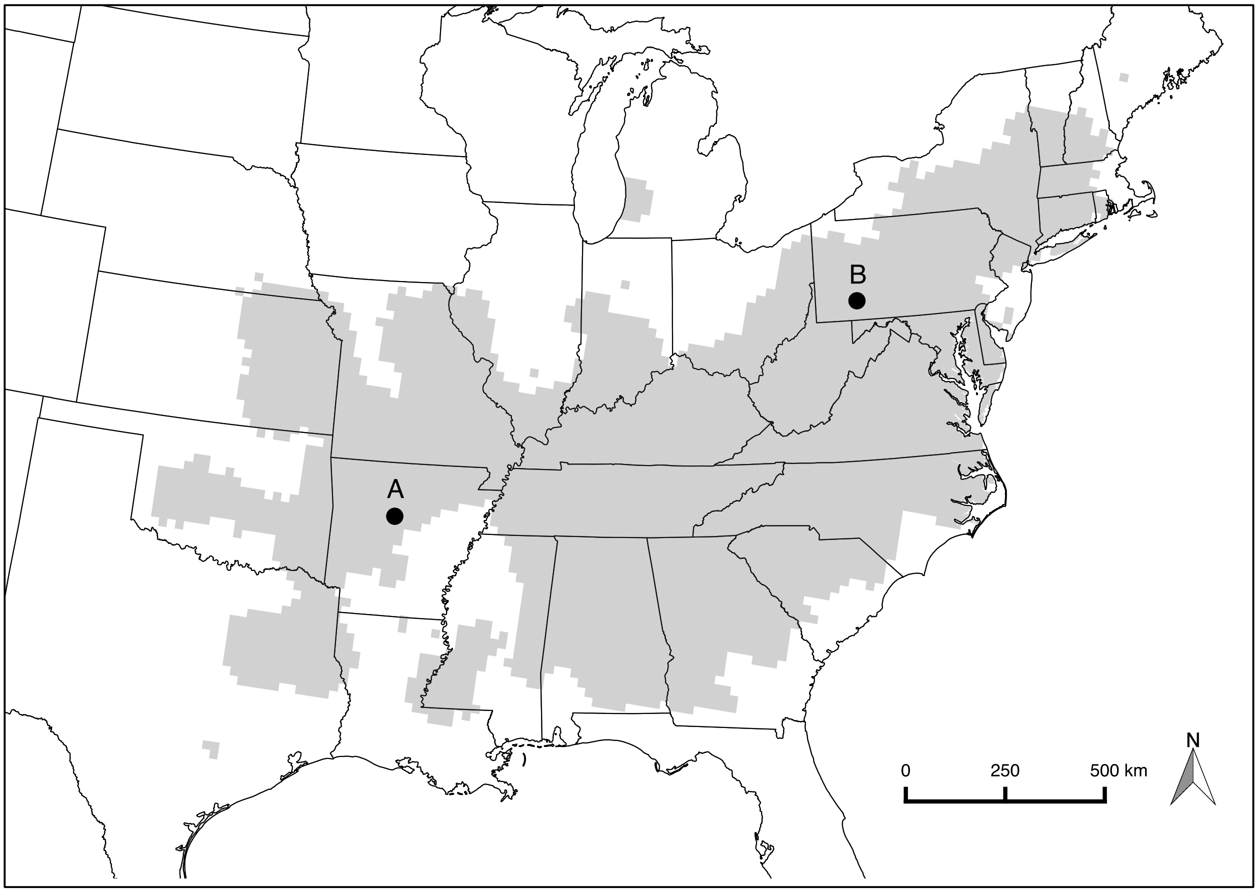

FIGURE 1.1. Location of study sites within the breeding range of Louisiana Waterthrush. (A) Study sites in Conway and Van Buren counties, Arkansas, and (B) Westmoreland County, Pennsylvania. Louisiana Waterthrush breeding range (shading) based on data from the North American Breeding Bird Survey (Sauer *et al.* 2014).

DNA Extraction, Amplification, and Sequencing

DNA was extracted from Louisiana Waterthrush nestling fecal samples using the QIAmp DNA Stool Mini Kit (Qiagen) and a customized protocol for avian fecal samples (Appendix A; Trevelline *et al.* 2016). Waterthrush fecal DNA was subjected to polymerase chain reaction (PCR) using the general arthropod ‘‘mini-barcode’’ primers ZBJArtF1c and ZBJ-ArtR2c, which amplify a 157 bp region of the *cytochrome c oxidase I* (COI) mitochondrial gene (Zeale *et al.* 2011). These primers were selected based on their ability to amplify degraded DNA and provide species-level taxonomic assignments from 13 arthropod orders (including EPT taxa; Zeale *et al.* 2011). Mini-barcode primers were modified by the addition of 5′ adapter sequences complementary to the Illumina multiplex indexing primers used in downstream sequencing protocols (Illumina 2013). PCR was conducted in 20 µL reactions with 10–100 ng of DNA template input, 4 µL of 5X high-fidelity reaction buffer (ThermoFisher Scientific), 400 µM dNTPs (ThermoFisher Scientific), 0.8 l µM modified forward primer ZBJ-ArtF1c (with 5′ adapter), 0.8 µM reverse primer ZBJ-ArtR2c (with 5′ adapter), and 0.1 units of Phusion Polymerase (Thermo-Fisher Scientific). All reactions were prepared on ice and amplified using the following conditions: an initial denaturation phase of 2 min at 98° C, 50 cycles of 10 s at 98° C, 30 s at 45° C, 30 s at 72° C, and a final extension of 10 min at 72° C. Amplification of the COI barcode was visually confirmed by ultraviolet trans-illumination following electrophoresis through a 2% agarose-ethidium bromide gel. Amplicons were enriched through an additional PCR reaction following the standard Illumina amplicon indexing and purification protocol (Illumina 2013). Indexed amplicons were combined at equimolar concentrations into a 250 bp, paired-end Illumina MiSeq sequencing run at the Genomics Facility of the Biotechnology Resource Center, Cornell University (Ithaca, NY).

Sequence Analysis

Sequences were quality trimmed in CLC Genomics Workbench 7.0.3 and filtered using Galaxy 15.10 (Blankenberg *et al.* 2010; Giardine *et al.* 2005; Goecks *et al.* 2010). Once trimmed of primers and adapters, any sequences that deviated from the expected amplicon size of 157 bp were removed from the analysis. All retained sequences exhibited a mean Phred quality score ≥ 30, which translates to a base-call error rate of 1 per 1000 bases (Ewing & Green 1998; Richterich 1998)

Filtered sequences were clustered into molecular operational taxonomic units (MOTUs) based on 97% similarity (appropriate for insects as discussed in Clare *et al.* 2011) using the bioinformatics program QIIME 1.8.0 (Caporaso *et al.* 2010). After excluding MOTUs with infrequent haplotypes (≤ 10 copies), representative sequences for each MOTU were compared to reference sequences in the Barcode of Life Database (BOLD; Ratnasingham & Hebert 2007). To ensure an accurate description of Louisiana Waterthrush diet from short fragments (157 bp) of the full-length (658 bp) COI barcode region (Hebert et al. 2003), only MOTUs that exhibited 100% similarity to a BOLD reference sequence were included in subsequent analyses (Appendix B; discussed in Clare *et al.* 2011).

The number of reads assigned to each successfully identified MOTU in a fecal sample was transformed into a presence or absence dataset. Louisiana Waterthrush nestling diet was summarized at the order-level based on the frequency of occurrence (number of fecal samples in which an order was detected divided by the total number of fecal samples) for each sampling region and time interval (e.g., Bowser *et al.* 2013; Razgour *et al.* 2011). This analysis approach is necessary for DNA metabarcoding studies because the proportion of sequencing reads within a sample does not necessarily reflect the relative quantities of prey consumed (Deagle *et al.* 2010; Pompanon *et al.* 2012).

Tests of statistical significance across nestling diets were calculated in R using a 2-sample proportion test (function: prop.test, alternative = two.sided). Nestling diet was summarized at the order-level in the program MEGAN 5.10.6 (Huson *et al.* 2011) based on the number of MOTUs that matched a BOLD reference sequence at 100%. Species accumulation curves and asymptotic species richness estimates were generated in R 3.2.2 using the library vegan (functions: specaccum, method = exact; poolaccum, index = chao; Oksanen *et al.* 2017).

1.3 RESULTS

Field Sampling

Louisiana Waterthrush nestling fecal samples were collected from nests along all study streams in both Arkansas (16) and Pennsylvania (16; see supplemental data in Trevelline *et al.* 2016). Sample collection dates were similar between Arkansas (May 14–June 19, 2013) and Pennsylvania (May 15–June 24, 2013) study regions. We collected 48 fecal samples from nestlings in Arkansas and 82 in Pennsylvania. One nest in Arkansas (3 fecal samples) and another in Pennsylvania (5 fecal samples) occurred uncharacteristically late in the breeding season (June 19 and June 24, respectively). Because these nests occurred beyond our analysis intervals, they were removed from our analysis of diet over the nesting period but remained part of our general description of Louisiana Waterthrush nestling diet (Table 1.1; Figures 1.2 and 1.3).

Benthic macroinvertebrates were collected in 2-week intervals from May 10 to July 7, 2013. Approximately 85% of subsampled benthic organisms were identified to the genus-level and represented 13 orders, which included EPT (see supplemental data in Trevelline *et al.* 2016). The mean relative abundance of EPT taxa was similar across study streams in Arkansas (0.60 ± 0.19) and Pennsylvania (0.72 ± 0.11; see supplemental data in Trevelline *et al.* 2016).

DNA Extraction, Amplification, and Sequencing

We successfully extracted DNA and amplified the COI barcode from all 130 Louisiana Waterthrush nestling fecal samples (Supplemental Table C.1, Appendix C). Template DNA concentrations ranged between 0.5 and 142.9 ng/µL with a mean of ~20 ng/µL. We successfully recovered sequence data from 123 fecal samples (95%). After quality trimming and the exclusion of infrequent haplotypes, we recovered 91,765 sequences that clustered into 125 (Arkansas) and

TABLE 1.1. Taxonomic assignment of molecular operational taxonomic units (MOTUs) detected in the diet of Louisiana Waterthrush nestlings in Arkansas and Pennsylvania. All listed taxa exhibited 100% similarity to a reference sequence in the Barcode of Life Database (BOLD). Frequency of occurrence = number of fecal samples (from a study region) in which an order was detected divided by the total number of fecal samples (from the same study region).

Class	Order	Family	Genus	Species	% Frequency of Occurrence (Arkansas)	% Frequency of Occurrence (Pennsylvania)
Arachnida	Araneae	Agelenidae	*Agelenopsis*	sp.	2.1	
		Anyphaenidae	*Anyphaena*	*pectorosa*		10.7
		Araneidae	*Eustala*	*anastera*	6.3	
			Larinioides	*cornutus*	4.2	
		Clubionidae	*Clubiona*	*canadensis*	4.2	4.0
		Linyphiidae	*Pityohyphantes*	*costatus*		5.3
		Salticidae	*Naphrys*	*pulex*		5.3
Insecta	Archaeognatha	Meinertellidae	*Machiloides*	*banksi*	10.4	
	Coleoptera	Carabidae	*Cyclotrachelus*	*sigillatus*		1.3
		Chrysomelidae	*Odontota*	*dorsalis*	8.3	
		Elateridae	*Athous*	*brightwelli*		16.0
				neacanthus		2.7
		Tenebrionidae	*Capnochroa*	*fuliginosa*		8.0
	Diptera	Asilidae	*Laphria*	*janus*	16.7	12.0
				prosticata		1.3
		Calliphoridae	*Calliphora*	*vomitoria*		10.7
			Phormia	*regina*		10.7
			Pollenia	*rudis*	45.8	14.7
		Empididae	*Rhamphomyia*	sp.	2.1	
		Limoniidae	*Epiphragma*	*fasciapenne*		2.7
			Eutonia	*alleni*	4.2	2.7
			Hexatoma	*spinosa*		9.3
		Pediciidae	*Tricyphona*	*inconstans*	4.2	5.3
		Scathophagidae	*Scathophaga*	*stercoraria*		1.3
		Syrphidae	*Syrphus*	*rectus*	14.6	22.7
				torvus	4.2	1.3
			Temnostoma	*alternans*		4.0
				balyras		4.0
		Tabanidae	*Chrysops*	*carbonarius*		2.7
				montanus		1.3
			Hybomitra	*lasiophthalma*		5.3
		Tipulidae	*Nephrotoma*	*virescens*	8.3	
			Tipula	*abdominalis*		1.3
				bicolor	8.3	
				fuliginosa	10.4	
				mallochi		17.3
				sp.	10.4	
	Ephemeroptera	Ameletidae	*Ameletus*	*lineatus*		13.3
		Baetidae	*Diphetor*	*hageni*		2.7
		Ephemerellidae	*Ephemerella*	*dorothea*		1.3
		Heptageniidae	*Epeorus*	*pleuralis*	41.7	48.0
			Heptagenia	sp.	43.8	45.3
			Maccaffertium	*meririvulanum*		2.7
				sp.	8.3	
				vicarium		9.3

TABLE 1.1. *Continued.*

Class	Order	Family	Genus	Species	% Frequency of Occurrence (Arkansas)	% Frequency of Occurrence (Pennsylvania)
	Hemiptera	Cicadellidae	*Gyponana*	sp.	2.1	
	Hymenoptera	Tenthredinidae	*Hemichroa*	*militaris*	4.2	
	Lepidoptera	Depressariidae	*Semioscopis*	*megamicrella*	4.2	
		Drepanidae	*Euthyatira*	*pudens*	2.1	1.3
			Habrosyne	*scripta*		4.0
		Erebidae	*Allotria*	*elonympha*	12.5	
			Catocala	*micronympha*	6.3	
				neogama	6.3	17.3
				sp.	4.2	
			Cissusa	*spadix*	10.4	
			Idia	*lubricalis*		1.3
			Lymantria	*dispar*	10.4	8.0
			Orgyia	*definita*	2.1	10.7
			Renia	*salusalis*	2.1	
			Zale	*minerea*	2.1	
		Geometridae	*Campaea*	*perlata*	4.2	5.3
			Epimecis	*hortaria*	14.6	17.3
			Eupithecia	*annulata*		1.3
			Heliomata	*cycladata*	2.1	
			Lomographa	*glomeraria*		26.7
			Melanolophia	*canadaria*		40.0
			Phigalia	sp.	16.7	
			Prochoerodes	*lineola*		2.7
			Tetracis	sp.	2.1	
		Lasiocampidae	*Malacosoma*	*disstria*		4.0
			Tolype	sp.	2.1	
		Noctuidae	*Achatia*	*distincta*	12.5	37.3
			Acronicta	*impleta*	4.2	
			Amphipyra	*pyramidoides*	2.1	
			Cerastis	*tenebrifera*	4.2	
			Eupsilia	*morrisoni*		9.3
				sp.	8.3	
			Helotropha	*leucostigma*	8.3	
			Lithophane	*unimoda*		16.0
			Metaxaglaea	*inulta*		5.3
				sp.	10.4	
			Morrisonia	sp.	12.5	
			Mythimna	*unipuncta*		1.3
			Orthosia	*garmani*	27.1	
			Orthosia	*hibisci*	10.4	2.7
			Sunira	*bicolorago*	8.3	2.7
			Xestia	sp.	8.3	
		Notodontidae	*Ellida*	*caniplaga*	4.2	4.0
			Heterocampa	*guttivitta*	2.1	
			Lochmaeus	*bilineata*	8.3	
				sp.	33.3	
			Nadata	*gibbosa*		6.7
		Tortricidae	*Acleris*	*nigrolinea*		1.3
			Phaecasiophora	*confixana*		1.3
			Pseudexentera	*oregonana*	2.1	
	Megaloptera	Corydalidae	*Nigronia*	*fasciatus*	16.7	20.0
		Sialidae	*Sialis*	sp.	6.3	6.7
	Orthoptera	Rhaphidophoridae	*Euhadenoecus*	*puteanus*		1.3
	Plecoptera	Perlidae	*Acroneuria*	sp.		6.7
		Perlodidae	*Isoperla*	*similis*		6.7
	Psocoptera	Peripsocidae	*Peripsocus*	*subfasciatus*		1.3
	Trichoptera	Limnephilidae	*Platycentropus*	*radiatus*		1.3

166 (Pennsylvania) MOTUs. Representative sequences (Supplemental Data D.1 and D.2, Appendix D) were compared to the BOLD reference library, which resulted in a 100% match to a reference sequence for 132 MOTUs (51,175 of recovered sequences) and 107 unique taxa (Table 1). Among these unique taxa, 83% were assigned to the species level and the remaining 17% to genus level (Table 1). We rejected 5 MOTUs because they were identified as Lepidoptera that do not occur in eastern North America (J. Rawlins, personal communication; see supplemental data in Trevelline *et al.* 2016). The order-level taxonomic richness of Louisiana Waterthrush nestling diet was similar in both Arkansas (9) and Pennsylvania (10; Figure 1.4, Panel A). By contrast, Arkansas waterthrush nestling diet exhibited substantially fewer MOTUs (58) compared to the diet of waterthrush nestlings in Pennsylvania (65; Figure 1.4, Panel B). Asymptotic species richness estimates at the MOTU-level suggest that the analysis of additional fecal samples may result in the identification of further prey taxa in both Arkansas (7 MOTUs) and Pennsylvania (14 MOTUs).

Waterthrush Nestling Diet

The terrestrial order Lepidoptera was detected in 92% of Louisiana Waterthrush nestling fecal samples and was significantly more common than all other orders except Diptera in Arkansas ($\chi^2 = 14.64$, df = 1, $P < 0.001$) and all other orders in Pennsylvania ($\chi^2 = 13.73$, df = 1, $P < 0.001$; Figure 1.2). Orders Diptera (70%) and Ephemeroptera (61%) were also frequently detected in both study regions (Figure 1.2). Among EPT taxa, Ephemeroptera was by far the most abundant, contributing to 93% of EPT MOTUs in samples collected from both study regions combined (Table 1.1, Figure 1.3). The mayfly family Heptageniidae was particularly well represented across fecal samples from both Arkansas (58%) and Pennsylvania (61%) and was the only family of Ephemeroptera detected in the diet of waterthrush nestlings in Arkansas

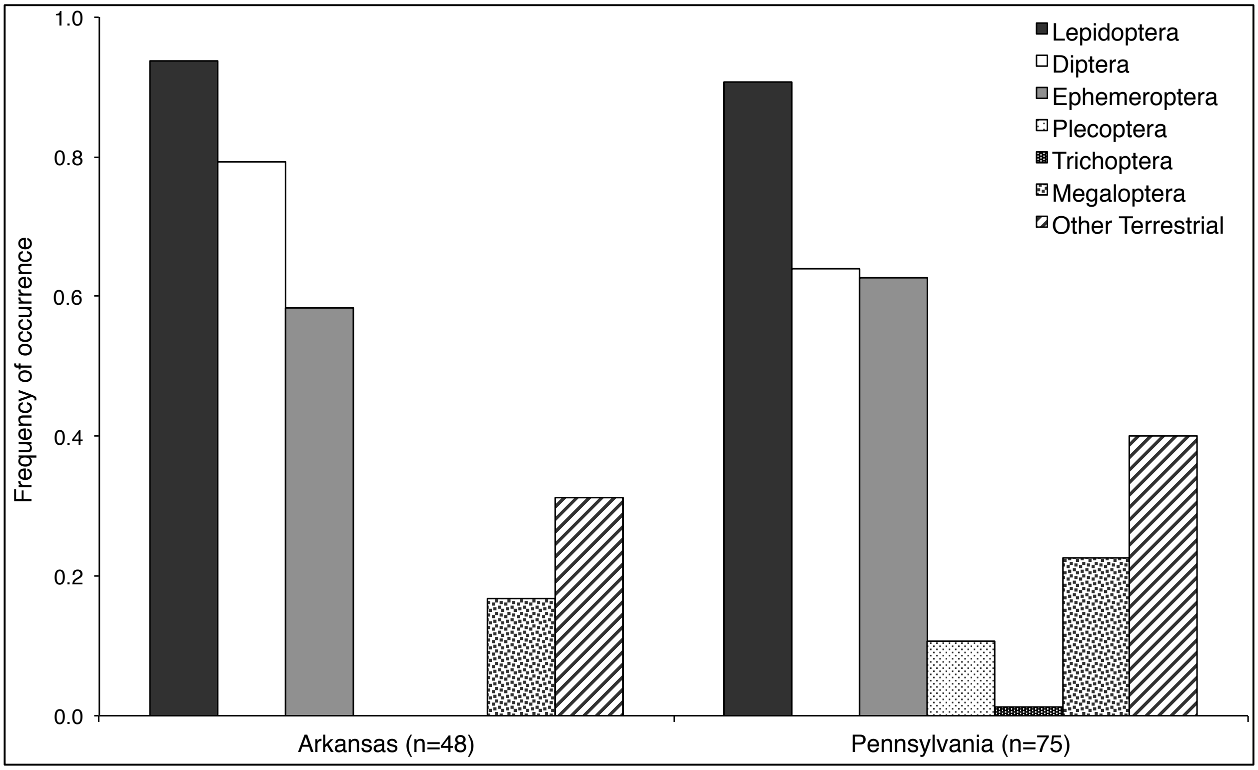

FIGURE 1.2. Frequency of occurrence of identified prey in the diet of Louisiana Waterthrush nestlings in Arkansas and Pennsylvania. The orders Lepidoptera (92%) and Diptera (70%) were the most common across waterthrush nestling fecal samples in both study regions. The order Ephemeroptera (60%) was detected frequently in both study regions while Plecoptera (7%) and Trichoptera (1%) were rarely detected. Frequency of occurrence = number of fecal samples (from a study region) in which an order was detected divided by the total number of fecal samples (from the same study region).

(Table 1). By contrast, 4 families of Ephemeroptera were found in waterthrush nestling diet in Pennsylvania: Ameletidae (13%), Baetidae (3%), Ephemerellidae (1%), and Heptageniidae (61%; Table 1). Orders Plecoptera (7%) and Trichoptera (1%) were detected in only 9 waterthrush fecal samples from Pennsylvania and were not detected in any fecal samples collected from Arkansas. Relaxing our conservative 100% similarity requirement to a less stringent ≥ 98% (Appendix B) did not result in additional detections of Plecoptera or Trichoptera (see supplemental data in Trevelline *et al.* 2016). In addition to the aquatic order Megaloptera (20%), several terrestrial orders were detected infrequently and analyzed as a group: Araneae, Archaeognatha, Coleoptera, Hemiptera, Hymenoptera, Orthoptera, and Psocoptera (Table 1.1, Figure 1.2).

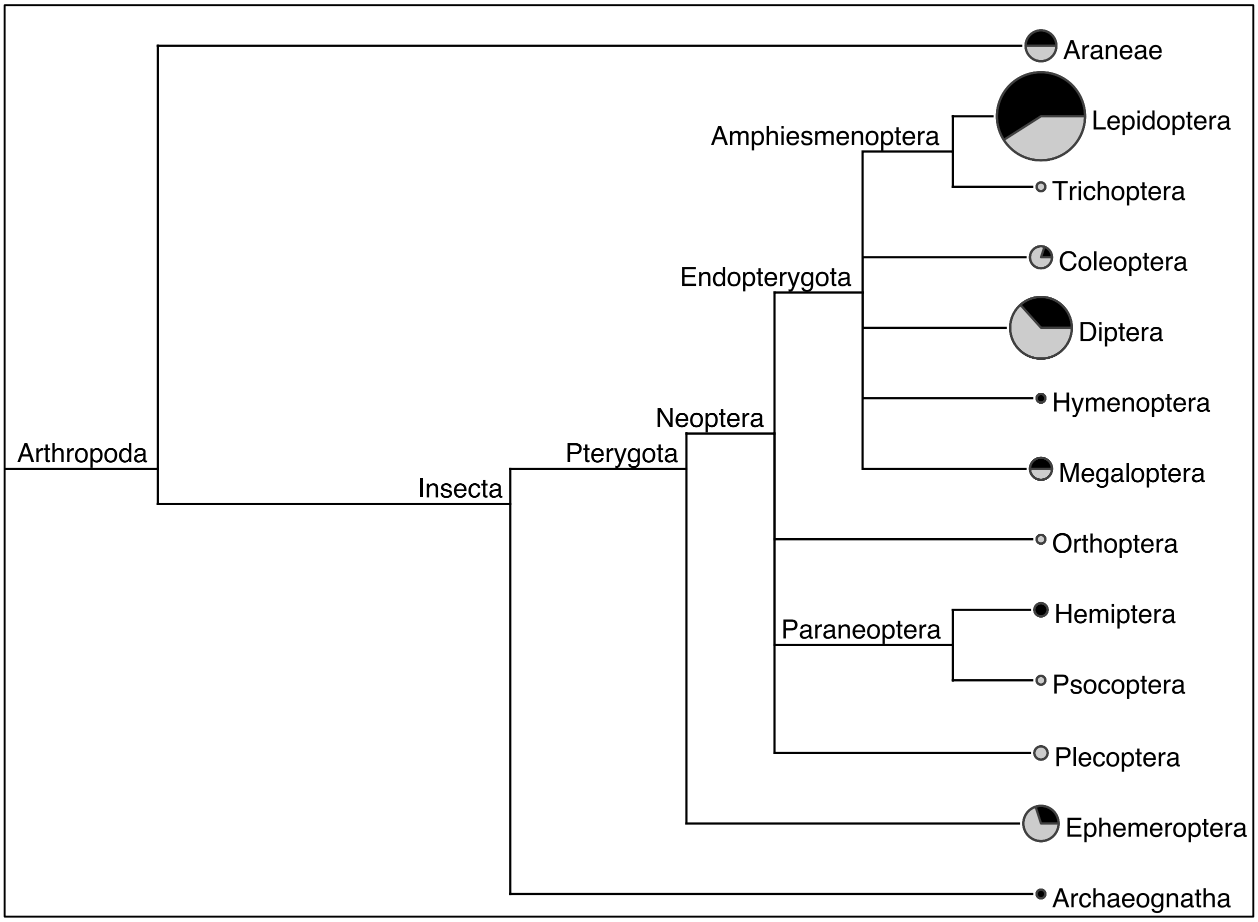

FIGURE 1.3. Order-level summary of Louisiana Waterthrush nestling diet in Arkansas and Pennsylvania. Tree includes MOTUs that exhibit 100% similarity to a reference sequence in BOLD for Louisiana Waterthrush fecal samples collected from Arkansas (black) and Pennsylvania (gray). Node size scaled to represent the number of identified MOTUs within a given order.

Based on our general description of waterthrush nestling diet (Figures 1.2 and 1.3), we investigated potential changes in frequency of occurrence over the nesting period for the 3 most commonly detected dietary orders: Lepidoptera, Diptera, and Ephemeroptera. In fecal samples collected from Arkansas, the frequency of occurrence of Lepidoptera ($\chi^2 < 0.01$, df = 1, $P > 0.05$) and Ephemeroptera ($\chi^2 < 0.45$, df = 1, $P > 0.05$) did not change over the course of the nesting period (Figure 1.5, Panel A). By contrast, among fecal samples collected from Pennsylvania, frequency of occurrence of Lepidoptera and Ephemeroptera differed significantly within the time intervals of late-May ($\chi^2 = 13.29$, df = 1, $P < 0.001$) and early-June

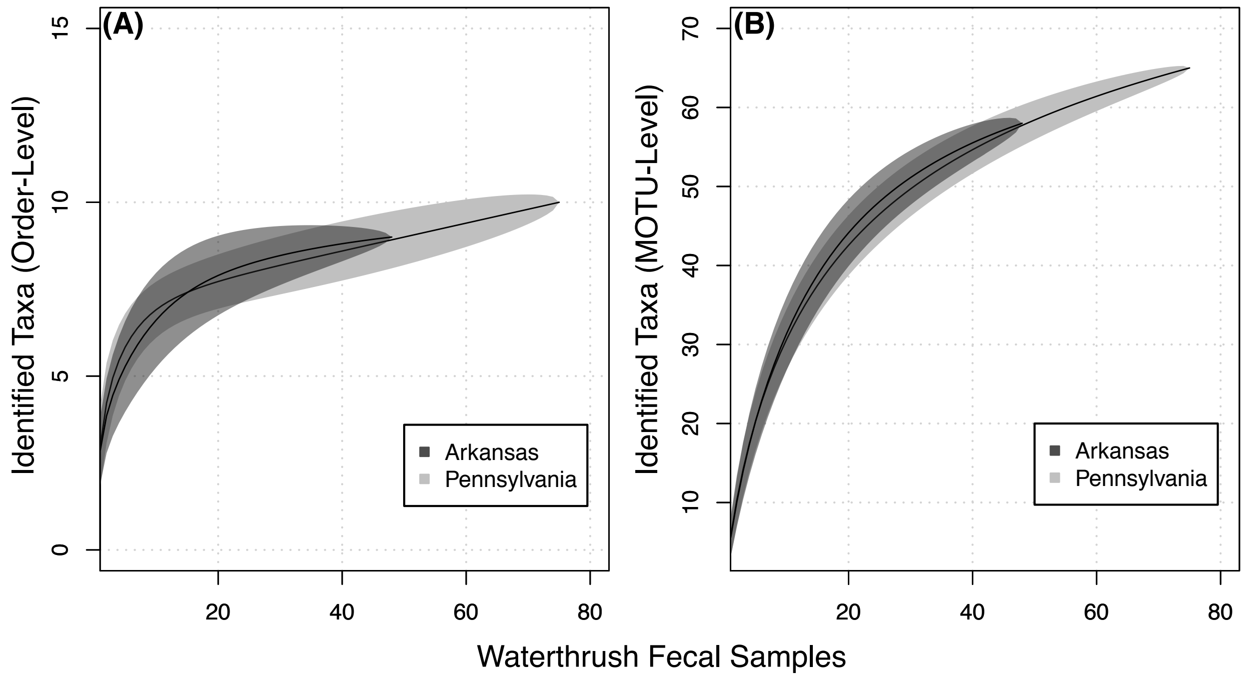

FIGURE 1.4. Species accumulation curves for the diversity of identified prey consumed by Louisiana Waterthrush nestlings at the (A) order-level and (B) MOTU-level. Lines represent mean estimates of taxon richness and shading represents standard deviation.

(χ^2 = 9.67, df = 1, P < 0.01). Furthermore, the frequency of occurrence for Ephemeroptera differed significantly (χ^2 = 6.82, df = 1, P < 0.01) over the course of the nesting period in Pennsylvania (Figure 1.5, Panel B). The order Diptera was also analyzed over these time intervals but did not differ significantly over the nesting period in Arkansas (χ^2 = 1.55, df = 1, P > 0.05) or Pennsylvania (χ^2 = 0.22, df = 1, P > 0.05; Figure 1.5).

1.4. DISCUSSION

We applied a next-generation sequencing approach to successfully identify Louisiana Waterthrush prey taxa to the genus or species level and elucidated the nestling diet of this Neotropical migrant. We found that waterthrush nestlings frequently consumed terrestrial Lepidoptera and Diptera in both study regions, contrary to the longstanding assertion that this species relies heavily on pollution-sensitive aquatic insects throughout its breeding range (Mattsson *et al.* 2009). The frequent detection of Lepidoptera and Diptera suggests that adult

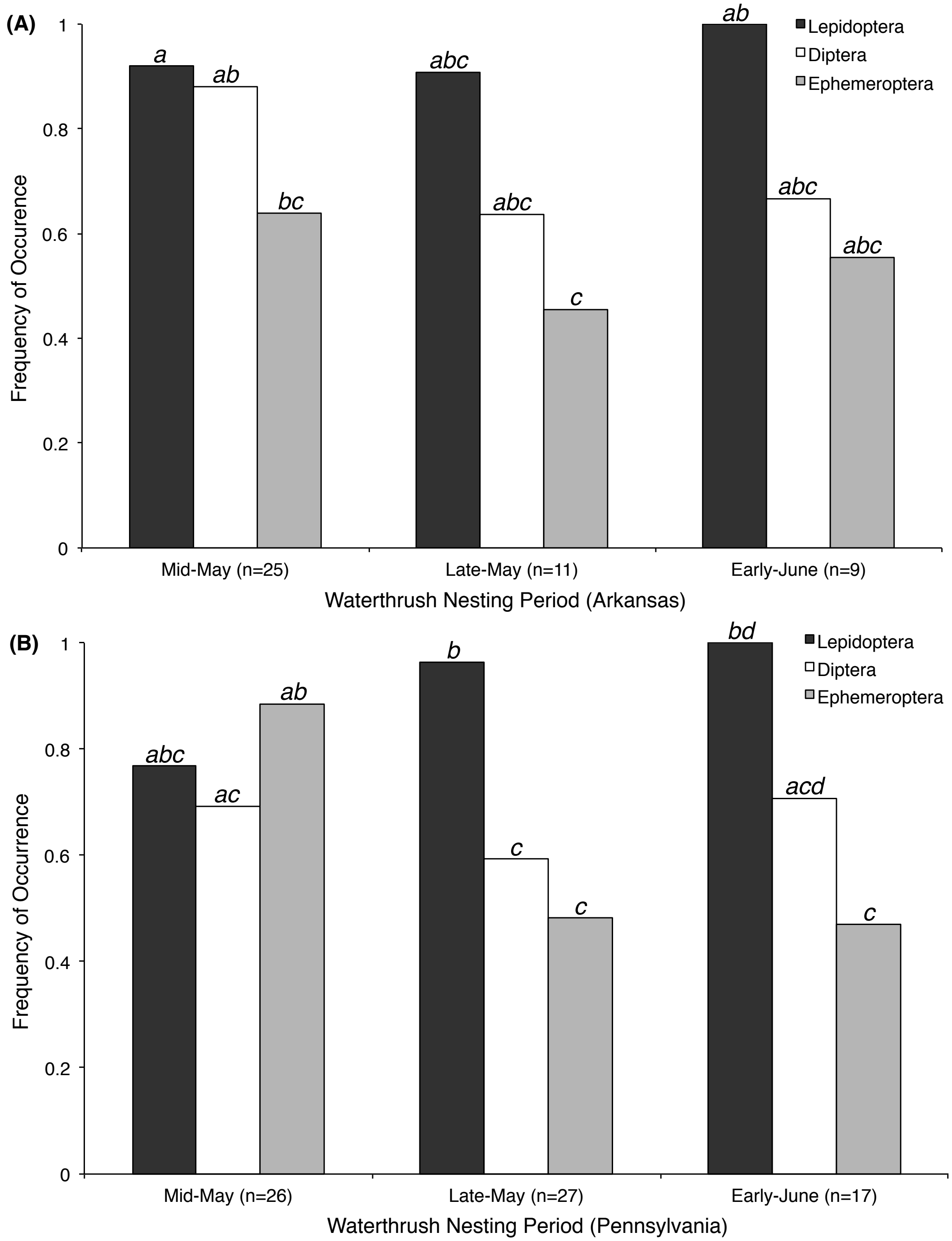

FIGURE 1.5. Frequency of occurrence of Lepidoptera, Diptera, and Ephemeroptera in the diet of Louisiana Waterthrush nestlings over the course of the nesting period in Arkansas and Pennsylvania. (A) In Arkansas, the frequency of occurrence of Lepidoptera and Ephemeroptera did not differ significantly over the course of the breeding season ($P > 0.05$). (B) In Pennsylvania, the frequency of occurrence of Lepidoptera and Ephemeroptera differed significantly within the late-May ($P < 0.001$) and early-June ($P < 0.01$) time intervals and over the course of the nesting period ($P < 0.01$). The order Diptera did not differ significantly over the nesting period in Arkansas or Pennsylvania ($P > 0.05$). Same letters above bars indicate no significant difference ($P > 0.05$). Frequency of occurrence = number of fecal samples (from a time interval) in which an order was detected divided by the total number of fecal samples (from the same time interval).

Louisiana Waterthrush target terrestrial taxa regularly, and that soft-bodied prey may have been overlooked in previous diet studies. Contrary to our hypothesis that EPT taxa would dominate waterthrush nestling diet, only the order Ephemeroptera was detected frequently. Plecoptera and Trichoptera were poorly represented despite their availability throughout waterthrush foraging territories in both Arkansas and Pennsylvania (see supplemental data in Trevelline *et al.* 2016), suggesting these taxa may not be important prey during the post-incubation period. These results were remarkably similar between study regions, which are ~ 1,300 km apart and on opposite extremes of the Louisiana Waterthrush breeding range (Figure 1.1).

The description of Louisiana Waterthrush diet presented here represents an account of prey taxa targeted by adults during the post-incubation period. Given previous research on waterthrush foraging behavior (Craig 1984; Eaton 1958; Mattsson *et al.* 2009), the large proportion of nestlings that consumed Lepidoptera (92%) and Diptera (70%) was unexpected. However, Louisiana Waterthrush have been observed to feed larval and adult Lepidoptera to nestlings at several of our study sites in Pennsylvania (R. Mulvihill, personal communication). Although differentiating between larval and adult life stages based solely on insect DNA is impossible, previous observational studies have reported that ~11% of Louisiana Waterthrush foraging was directed at riparian foliage during the post-incubation period (Mattsson *et al.* 2009). Foliage serves as a host for larval Lepidoptera, which have been suggested as an important food item for the nestlings of other Neotropical migrants (Holmes *et al.* 1979). Clearly, the high frequency of detection for orders Lepidoptera and Diptera suggests that non-EPT taxa may be more important to Louisiana Waterthrush than previously thought. This finding emphasizes the need for improved understanding of Louisiana Waterthrush foraging ecology and how changes in the availability of non-EPT taxa influence both nestlings and adults.

In Pennsylvania, we found that Louisiana Waterthrush nestling diet changed over the course of the nesting period. This shift in diet resulted from a significant reduction in the detection of dietary Ephemeroptera and an increased detection of Lepidoptera in the later stages of the nesting period, suggesting that a reduction in the availability of Ephemeroptera or an increased availability of Lepidoptera may be driving the change in diet. Louisiana Waterthrush may therefore target Ephemeroptera in the early season but switch to Lepidoptera as they become available later in the breeding season. This shift was not observed in the diet of waterthrush nestlings in Arkansas, which may be partly explained by the phenology of waterthrush. Neotropical migrants are believed to rely on photoperiod cues to determine date of departure from the wintering grounds (Hagan *et al.* 1991) to maximize phenological synchrony and the availability of insects during chick rearing (Lany *et al.* 2015; Perrins 1970). Yet latitudinal and climatic differences across the Louisiana Waterthrush breeding range affect the timing of leaf expansion and Lepidoptera prey abundance (e.g., Butler & Strazanac 2000; Parry *et al.* 1998). Therefore, we might expect Lepidoptera to be available prey earlier in the breeding season for waterthrush in Arkansas than for conspecifics nesting in Pennsylvania. Our findings suggest that the availability of terrestrial prey such as Lepidoptera and Diptera may be important to Louisiana Waterthrush during the post-incubation period and should be a priority for future research. These results also emphasize the plasticity of waterthrush diet, but whether changes in the orders of prey insects consumed affect waterthrush nest success or other vital rates remains unknown.

Despite the frequent detection of Lepidoptera in nestling diet, previous studies have convincingly demonstrated that Louisiana Waterthrush respond negatively to reductions in EPT availability (Mattsson & Cooper 2006; Mulvihill *et al.* 2009; Mulvihill *et al.* 2008; Wood *et al.*

2016). EPT taxa are also reliable indicators of overall riparian quality (Barbour *et al.* 1999; Hilsenhoff 1977) and reflect several factors that impact the suitability of waterthrush breeding territories (e.g., bank erosion, anthropogenic land use, and stream order; Brooks *et al.* 1998; Mattsson & Cooper 2006; Prosser & Brooks 1998). Therefore, EPT taxa may be a reliable indicator of waterthrush site occupancy but may not completely reflect their foraging ecology. As predicted by a previous study (Mulvihill *et al.* 2008), we found that Ephemeroptera (61%) were particularly well-represented across Louisiana Waterthrush diets. Whether those prey individuals were larval (aquatic) or adult (terrestrial) Ephemeroptera remains unknown and represents an important limitation of molecular diet analyses. Regardless, the frequency of occurrence of a single family of Ephemeroptera (Heptageniidae) in waterthrush nestling fecal samples (60%) is particularly interesting because it contains several of the most pollution-sensitive aquatic insects in eastern North America (Barbour *et al.* 1999). Reliance on Heptageniidae raises considerable conservation concern as anthropogenic impacts to water quality continue throughout the Louisiana Waterthrush breeding range (Drohan *et al.* 2012; Wood *et al.* 2016).

Our results were derived using a single primer set designed to amplify a small fragment (157 bp) of a single barcode marker (COI) and should not be considered a comprehensive description of Louisiana Waterthrush nest ling diet. To confidently identify all dietary insects, our methodology should be expanded to include multiple primer sets or additional barcoding genes, which may capture a greater variety of prey taxa (Bowser *et al.* 2013; Hajibabaei *et al.* 2012). Unfortunately, the potential advantages of alternative barcoding markers for insectivores are hindered by a relatively limited barcode library compared to that currently available for COI. Furthermore, the arthropod COI barcode library managed by BOLD is ideal because of strict vouchering requirements that reduce the risk of misidentification (Ratnasingham & Hebert 2007). The application of a single primer set (Zeale *et al.* 2011) is not expected to have biased our results however, as demonstrated by several studies that also identified EPT taxa using the same primer set (e.g., Clare *et al.* 2009; Razgour *et al.* 2011; Vesterinen *et al.* 2013); therefore, the use of a single primer set and genetic marker should not diminish the conclusions of this study.

Until now, our understanding of Louisiana Waterthrush nestling diet was limited to studies that used morphological identification (Eaton 1958) and foraging observations of adults (Craig 1984). We now understand that waterthrush nestling diet is broader than previously thought and includes non-EPT taxa such as terrestrial Diptera and Lepidoptera. Although most of our analyses were collapsed to the order-level, we identified soft-bodied prey taxa (orders Diptera and Lepidoptera) that may have escaped detection using morphological identification techniques. These findings demonstrate the advantages of DNA-based techniques for studying the diet of Neotropical migrants and emphasize the need for its widespread application. Our results may be particularly interesting to ecologists studying species with similar foraging specialties or limited dietary information. The incomplete understanding of Neotropical migrant diet is a pervasive problem, but with the advent of DNA-based approaches, ornithologists are now able to investigate some of the most elusive questions regarding the importance of diet throughout the annual cycle.

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CHAPTER TWO

DNA metabarcoding of nestling feces reveals provisioning of aquatic prey and resource partitioning among Neotropical migratory songbirds in a riparian habitat

Riparian habitats are characterized by substantial flows of emergent aquatic insects that cross the stream-forest interface and provide an important source of prey for insectivorous birds. The increased availability of prey arising from aquatic subsidies attracts high densities of Neotropical migratory songbirds that are thought to exploit emergent aquatic insects as a nestling food resource; however, the prey preferences and diets of birds in these communities are only broadly understood. In this study, we utilized DNA metabarcoding to investigate the extent to which three syntopic species of migratory songbirds—Acadian Flycatcher, Louisiana Waterthrush, and Wood Thrush—breeding in Appalachian (Pennsylvania, USA) riparian habitats exploit and partition aquatic prey subsidies as a nestling food resource. Despite substantial differences in adult foraging strategies, nearly every nestling in this study consumed aquatic taxa, suggesting that aquatic subsidies are an important prey resource for Neotropical migrants nesting in riparian habitats. While our results revealed significant interspecific dietary niche divergence, the diets of Acadian Flycatcher and Wood Thrush nestlings were strikingly similar and exhibited significantly more overlap than expected. These results suggest that the dietary niches of Neotropical migrants with divergent foraging strategies may converge due to the opportunistic provisioning of non-limiting prey resources in riparian habitats. In addition to providing the first application of DNA metabarcoding to investigate diet in a community of Neotropical migrants, this study emphasizes the importance of aquatic subsidies in supporting breeding songbirds and improves our understanding of how anthropogenic disturbances to riparian habitats may negatively impact long-term avian conservation.

2.1 INTRODUCTION

As the interface between two biomes, streamside riparian habitats are characterized by a substantial flow of organic materials that cross the aquatic-terrestrial boundary and provide important resource subsidies for consumers (Baxter *et al.* 2005; Polis *et al.* 1997). While it has long been recognized that allochthonous inputs of leaves, woody debris, and insect larvae from the surrounding forest are essential for stream biota (Nakano *et al.* 1999; Vannote *et al.* 1980; Wallace *et al.* 1997), recent attention has highlighted that emergent aquatic insects provide an important reciprocal subsidy for riparian insectivores (reviewed in Baxter *et al.* 2005). Terrestrial predators functionally and numerically respond to increased prey availability arising from aquatic subsidies, resulting in a more diverse and densely populated community of insectivores compared to adjacent non-riparian habitats (Baxter *et al.* 2005). This phenomenon is particularly evident among breeding Neotropical migratory songbirds (Gray 1993; Hodges & Krementz 1996; Whitaker *et al.* 2000), many of which have experienced long-term population declines (Robbins *et al.* 1989; Sauer *et al.* 2014; Sauer & Link 2011). Thus, riparian habitats throughout North America are exceptionally valuable to avian conservation (Knopf *et al.* 1988; Knopf & Samson 1994; Saab 1999).

The availability of insect prey during breeding has been identified as a major limiting factor for Neotropical migratory songbird populations (reviewed in Martin 1987; Newton 2004). Experimental manipulations of larval Lepidoptera availability (frequently targeted by Neotropical migrants; Holmes *et al.* 1979b) have demonstrated that prey limitations can strongly influence reproductive output by negatively impacting clutch initiation (Marshall *et al.* 2002), clutch size (Rodenhouse & Holmes 1992), nestling survival (Nagy & Smith 1997), and number of nesting attempts (Nagy & Holmes 2005). For Neotropical migrants breeding in riparian habitats, emergent aquatic insects represent a considerable proportion of available prey (Nakano & Murakami 2001) and are thought to be an important food resource for both adults (e.g., Busby & Sealy 1979; Raley & Anderson 1990) and nestlings (e.g., Biermann & Sealy 1982; Wiesenborn & Heydon 2007). Similar to the documented impacts of food limitations on Neotropical migrants breeding in upland forests (reviewed in Newton 2004), the reduced availability of aquatic insect prey in riparian habitats (primarily due to anthropogenic stream acidification) has been shown to negatively impact factors critical to the breeding productivity of songbirds such as clutch initiation (Mulvihill *et al.* 2008), clutch size (Ormerod *et al.* 1991), nestling body condition (O'Halloran *et al.* 1990), risk of nestling predation (O'Halloran *et al.* 1990), nestling survival (Vickery 1992), and the number of nesting attempts (Mulvihill *et al.* 2009).

Despite the potentially negative impact of prey limitations on their long-term conservation, the diets of Neotropical migratory songbirds remain only broadly understood. This knowledge gap is primarily due to the coarse taxonomic resolution (typically order or family; Rosenberg & Cooper 1990) of traditional morphological approaches that describe diets using insect remains from stomach contents (e.g., Rosenberg *et al.* 1982) and fecal samples (e.g., Wiesenborn & Heydon 2007). The morphological identification of prey from feces is especially problematic for studying the diets of Neotropical migrant nestlings, which are thought to primarily consume soft-bodied insects (e.g., Diptera and larval Lepidoptera; Biermann & Sealy 1982; Holmes *et al.* 1979b) that are difficult to identify after digestion (Rosenberg & Cooper 1990). Because nestling diets provide insights into adult foraging behavior during nest provisioning, these limitations present a considerable barrier to understanding how Neotropical migratory songbird communities exploit and partition prey resources during one of the most energetically demanding (Holmes *et al.* 1979a) and critical (reviewed in Martin 1987) periods of the annual cycle. For example, resource partitioning theory predicts that prey utilization will differ between syntopic species in order to limit competition (e.g., Cody 1968; Schoener 1974), but order-level dietary descriptions using traditional morphological techniques are unlikely to resolve subtle (but potentially significant) differences in prey utilization. In contrast, genus- or species-level dietary descriptions are capable of distinguishing such differences in diet composition (e.g., Krüger *et al.* 2014), and thus may improve our understanding of how songbirds partition prey resources and minimize competition within the dense breeding communities of riparian habitats. Furthermore, this level of taxonomic resolution may reveal preferences for specific aquatic taxa that differ greatly in their life-histories, emergence patterns, and tolerances to stream contamination (Barbour *et al.* 1999; Merritt & Cummins 2008). Given the documented impacts of prey limitations on the breeding productivity of Neotropical migratory birds, this knowledge gap presents a problematic barrier to understanding how current (reviewed in Dudgeon *et al.* 2006) and future (e.g., Drohan *et al.* 2012) anthropogenic disturbances to stream habitats will influence the long-term conservation of avian diversity in riparian ecosystems.

The identification of prey from animal feces using a combination of DNA barcoding and next-generation sequencing (hereafter DNA metabarcoding) is increasingly utilized for the study of predator diets. This approach can be applied to a wide range of diet types (Pompanon *et al.* 2012) and has distinct advantages over traditional morphological analyses such as species-level taxonomic resolution (e.g., Trevelline *et al.* 2016) and non-invasive sampling (ideal for study species of conservation concern; Clare 2014). Despite widespread application that has resulted in an improved understanding of trophic ecology across most major taxonomic groups (see

Symondson & Harwood 2014), DNA metabarcoding has rarely been applied to the diets of passerine birds (order Passeriformes), which represent over 50% of extant avian taxa (Jetz *et al.* 2012). In the limited number of studies that have utilized DNA metabarcoding to investigate the diets of passerines (only 3 studies to date; Crisol-Martínez *et al.* 2016; Jedlicka *et al.* 2016; Trevelline *et al.* 2016), this approach has revealed the consumption of insects not previously known to be prey for their respective focal species. For example, Crisol-Martínez *et al.* (2016) and Jedlicka *et al.* (2016) successfully demonstrated that insectivorous birds consumed several species of herbivorous insects in agricultural landscapes, thus providing valuable pest-reduction services in agro-ecosystems. In the only application of DNA metabarcoding to the diet of a Neotropical migratory songbird, Trevelline *et al.* (2016) demonstrated that the nestlings of the stream-dependent Louisiana Waterthrush (*Parkesia motacilla*) regularly consumed terrestrial Lepidoptera, which may have escaped detection in previous diet studies that relied on traditional morphological approaches.

In this study, we utilized DNA metabarcoding to investigate the extent to which a suite of breeding Neotropical migratory songbirds exploit and partition aquatic prey subsidies in riparian habitats. To accomplish this, we studied nestling diets in a riparian community consisting of three syntopic species with marked differences in foraging strategies. We hypothesized that (1) aquatic prey taxa would be a major component of nestling diets, and (2) nestling species would occupy distinct dietary niches that reflect documented differences in foraging strategies.

2.2 MATERIALS AND METHODS

Study species and sample collection

We focused on three syntopic species of insectivorous Neotropical migrant songbirds that commonly breed in the riparian zones of southwestern Pennsylvania: Acadian Flycatcher (*Empidonax virescens*), Louisiana Waterthrush (*Parkesia motacilla*), and Wood Thrush (*Hylocichla mustelina*). While it is important to note that these focal species are experiencing population declines throughout their respective Appalachian ranges (Sauer *et al.* 2014), they were primarily selected based on their abundance at our field site and divergent foraging strategies that maximized the likelihood of differential aquatic prey utilization.

The Louisiana Waterthrush is an obligate riparian wood-warbler (family Parulidae) that nests directly in stream banks and primarily forages at ground-level for aquatic insects (both larval and adult) along stream edges (~ 90% of foraging maneuvers directed at water; Mattsson *et al.* 2009). The Acadian Flycatcher (family Tyrannidae) typically nests in tree branches overhanging headwater streams and captures flying insects (which may have aquatic larval stages) from an elevated perch (Whitehead & Taylor 2002). The Wood Thrush (family Turdidae) can nest in a variety of understory vegetation types (in both riparian and upland habitats) and forages primarily on the ground for terrestrial insects occurring in the leaf litter (Evans *et al.* 2011). All three of these species have been reported to occasionally glean insects from foliage (Evans *et al.* 2011; Mattsson *et al.* 2009; Whitehead & Taylor 2002).

Nests of focal species were systematically located and monitored within a 100-meter riparian buffer strip (~ 2 km in length) along the mainstem of three headwater Appalachian streams near Powdermill Nature Reserve (Rector, Westmoreland County, PA) from April to July 2015: Laurel Run, Loyalhanna Creek, and Powdermill Run. Fecal samples were collected by placing nestlings (4-8 days old) into a clean paper bag (for a maximum of 3 minutes) or by encouraging voidance directly over an open vial. Each fecal sample was preserved in 20 mL of 100% ethanol and stored at -20°C for approximately 3 months prior to DNA extraction.

Molecular analysis and bioinformatics

Prey DNA was extracted from nestling fecal samples using the QIAmp DNA Stool Mini Kit (Qiagen) and a protocol optimized for avian feces (Trevelline *et al.* 2016). Fecal DNA extractions were subjected to polymerase chain reaction (PCR) using the universal arthropod COI "mini-barcode" primers ZBJ-ArtF1c and ZBJ-ArtR2c (Zeale *et al.* 2011), which were modified by the addition of a 5′ adapter sequence complementary to Illumina Nextera XT (v2) indexing primers (see Trevelline *et al.* 2016). PCR reactions (20 µL) were prepared according to Trevelline *et al.* (2016): 10-100 ng of template input, 4 µL of 5X high-fidelity reaction buffer (ThermoFisher Scientific), 400 µM dNTPs (ThermoFisher Scientific), 0.8 µM of ZBJ-ArtF1c (with 5′ adapter), 0.8 µM of ZBJ-ArtR2c (with 5′ adapter), and 0.1 units of Phusion Polymerase (ThermoFisher Scientific). PCR amplification of COI mini-barcodes was performed in duplicate for each fecal sample (e.g., Crisol-Martínez *et al.* 2016; Trevelline *et al.* 2016; but see justification for triplicate PCR in Vo & Jedlicka 2014) using the following conditions: an initial denaturation phase of 98 °C for 2 minutes; 50 cycles at 98 °C for 10 seconds, 45 °C for 30 seconds, and 72 °C for 30 seconds; a final extension phase of 72 °C for 10 minutes. Amplicons from duplicate reactions were pooled for an additional enrichment and indexing PCR using the Illumina Nextera XT (v2) Indexing Kit following the manufacturer's instructions. Once indexed, amplicons were pooled at equimolar concentrations for analysis (250 bp paired-end) using the Illumina MiSeq next-generation sequencing platform.

Raw Illumina sequence reads were trimmed and quality filtered (Phred ≥ 30) using the CLC Genomics Workbench 7.0.3 (Qiagen) and Galaxy 15.10 (Blankenberg *et al.* 2010; Giardine *et al.* 2005; Goecks *et al.* 2010). Filtered sequences were clustered into molecular operational taxonomic units (MOTUs) based on 97% similarity (ideal sequence divergence threshold for COI amplicons using ZBJ primers; Razgour *et al.* 2011) using QIIME 1.8.10 (pick_de_novo_otus.py; Caporaso *et al.* 2010). To conservatively describe riparian nestling diets and focus on the major dietary differences between species, MOTUs that occurred infrequently across fecal samples (< 5%) or consisted of rare sequence haplotypes (< 10 copies) were excluded from downstream analyses (Trevelline *et al.* 2016). Representative sequences from each MOTU were selected in QIIME (pick_rep_set.py; Caporaso *et al.* 2010), queried in the Barcode of Life Database (BOLD; Ratnasingham & Hebert 2007), and binned into 1 of 6 possible categories designed to prioritize sequences with genus or species-level taxonomic resolution and > 98% percent match to a reference sequence (see Trevelline *et al.* 2016 for detailed description of scoring criteria). To minimize the likelihood of taxonomic misidentifications from short fragments (157 bp) of the full-length (658 bp) COI barcode, MOTUs that exhibited < 98% similarity to a reference sequence or could not provide genus- or species-level resolution were classified as "unidentified" and excluded from taxonomic descriptions of diet (discussed in Clare *et al.* 2011). Because the proportion of sequencing reads does not necessarily reflect the relative quantities of prey consumed (Pompanon *et al.* 2012), the number of reads assigned to each dietary MOTU were transformed into a presence-absence dataset, which was subsequently used to calculate frequency of occurrence (number of fecal samples in which a MOTU was detected divided by the total number of fecal samples) for each nestling species (Trevelline *et al.* 2016).

Diet analysis

To assess interspecific differences in nestling dietary niche breadths, the frequency of occurrence of dietary MOTUs were used to calculate Levins' Index (reciprocal of Simpson's Index of diversity; Levins 1968) in the R package *vegan* (Oksanen *et al.* 2017; function: diversity, index = "invsimpson"). Levins' Index of dietary niche breadth was standardized based on the total number of MOTUs in the diets of riparian nestlings (all three species) to generate a value ranging from 0 to 1, where 1 represents a diet consisting of all detected MOTUs (Hurlbert 1978; see Razgour *et al.* 2011 for molecular diet application). To estimate the expected number of undetected MOTUs, dietary richness rarefaction curves were generated and extrapolated using the Chao method in the R package *iNEXT* (function: iNEXT, datatype = "incidence_freq"; Chao *et al.* 2014; Hsieh *et al.* 2016). Taxonomic dietary descriptions were summarized by frequency of occurrence for each nestling species at the order, family, and MOTU level. Identified dietary MOTUs with an aquatic larval stage (hereafter "aquatic prey taxa") and those without an aquatic larval stage (hereafter "terrestrial prey taxa") were classified as such using the genus-level life history characteristics provided by Merritt and Cummins (2008). Differences in the consumption of aquatic prey were based on the proportion of aquatic MOTUs and analyzed using an analysis of variance (ANOVA) with a random term to account for the clustering of nestling fecal samples collected from the same nest.

The frequency of occurrence of dietary MOTUs (including those that were unidentified; discussed in Clare *et al.* 2011) were used to calculate interspecific dietary niche overlap via Pianka's Index (Pianka 1973; see Razgour *et al.* 2011 for molecular diet application). To test the hypothesis that interspecific dietary niche overlap was greater than expected by chance, Pianka's Index (ranges from 0 to 1, where 1 represents complete diet overlap) was calculated relative to null models of randomized MOTU frequency of occurrence data in the R package *EcoSimR* (Gotelli *et al.* 2015; function: niche_null_model, algo = "ra3", metric = "pianka", nReps = 10,000).

To test the hypothesis that the dietary niches of nestlings differ between species, Jaccard distances (based on nest-level summaries of MOTUs to account for the clustering of nestling fecal samples collected from the same nest) were analyzed using a permutational multivariate analysis of variance (PERMANOVA; Anderson 2001; see Crisol-Martínez *et al.* 2016 for molecular diet application) in the R package *vegan* (Oksanen *et al.* 2017; function: adonis, method = “jaccard”, permutations = 999). Interspecific differences in diet variability were investigated using the multivariate homogeneity of group dispersions (Anderson 2006) for each nestling species in *vegan* (Oksanen *et al.* 2017; function: betadisper, group = “species”, type = “median”). Nestling dietary niches were visualized using non-metric multidimensional scaling (NMDS; Kruskal 1964) in *vegan* (Oksanen *et al.* 2017; function: metaMDS, distance = “jaccard”, k = 2), which generates a two-dimensional unconstrained ordination plot that illustrates compositional differences between individual diets while preserving the rank-order relationships in total multivariate diet space (see Krüger *et al.* 2014 for molecular diet application). The dietary niche space for each nestling species was visualized in *vegan* using minimum convex polygons (function: ordihull) and 95% confidence ellipses around species centroids (function: ordiellipse, kind = “se”, conf = 0.95).

2.3 RESULTS

COI barcodes were successfully retrieved from 134 nestling fecal samples representing a total of 43 nests (17 Acadian Flycatcher, 9 Louisiana Waterthrush, and 17 Wood Thrush;

Supplemental Table C.2, Appendix C). After quality filtering and trimming, Illumina sequencing generated 3,474,157 reads that clustered into 262 MOTUs after the removal of infrequent haplotypes (3,094,053 remaining sequences; mean of 23,090 per sample ± 12,426 SD). MOTU-level dietary richness was substantially lower for the nestlings of Louisiana Waterthrush (120) compared to the nestlings of Acadian Flycatcher (218) and Wood Thrush (237); however, Chao asymptotic richness estimates indicated the presence of several undetected MOTUs (mean = 11 ± 6 SD) in the diets of each focal species (Figure 2.1). Differences in MOTU-level dietary richness between nestling species were reflected by similar differences in Levins' Index of dietary niche breadth with Louisiana Waterthrush (0.22) exhibiting a much narrower dietary niche relative to Acadian Flycatcher (0.44) and Wood Thrush (0.51). Identification of MOTU representative sequences (Supplemental Data D.3, Appendix D) in the BOLD reference library resulted in a ≥ 98% match to genus or species for 132 MOTUs (~ 50% of total MOTUs) representing 120 unique dietary taxa (Table 2.1). Interspecific differences in MOTU-level dietary richness and Chao estimates using only identified taxa were similar to those observed using all MOTUs with Louisiana Waterthrush consuming substantially fewer dietary taxa (59) than Acadian Flycatcher (100) and Wood Thrush nestlings (107; Figure 2.1).

Overall, 15 orders and 56 families of arthropods were detected across nestling diets (Figure 2.2; Table 2.2). Lepidoptera was the most frequently detected arthropod order across nestling diets (99%; Table 2.2) with the terrestrial families Erebidae (67%), Geometridae (70%), and Noctuidae (96%) being the most common (Figure 2.2). The order Diptera was also frequently detected across nestling diets (95%; Table 2.2), but terrestrial taxa in this order were rarely consumed by Louisiana Waterthrush nestlings (Figure 2.2). Similarly, terrestrial taxa in the orders Coleoptera and Araneae were frequently detected in the diets of Acadian Flycatcher

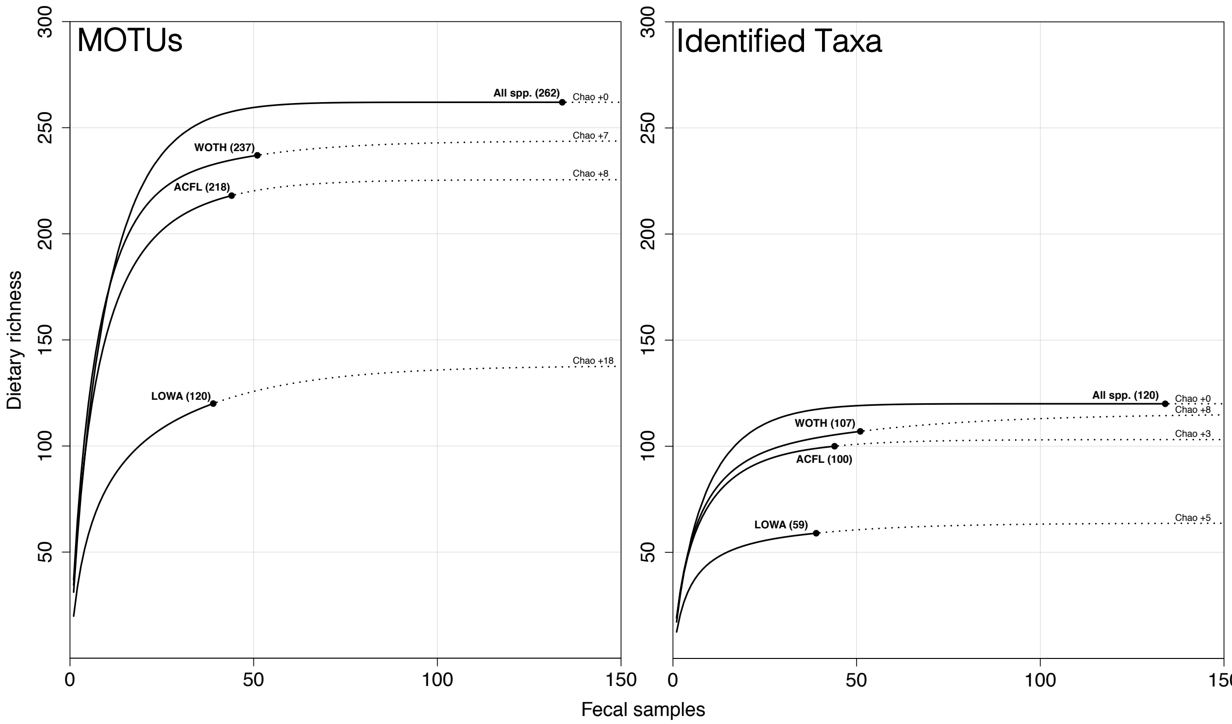

FIGURE 2.1. Rarefaction curves of MOTUs (left) and identified prey taxa only (right) in the diets of Acadian Flycatcher (ACFL; n = 44), Louisiana Waterthrush (LOWA; n = 39), and Wood Thrush (WOTH; n = 51) nestlings. Solid lines represent mean Chao richness estimates based on permutations and points indicate observed dietary richness for each nestling species. Dotted lines represent extrapolated Chao richness estimates and annotations indicate the expected number of additional dietary MOTUs (left) and identified taxa (right) for each nestling species.

and Wood Thrush nestlings, but were rare or absent in Louisiana Waterthrush nestling diets (Figure 2.2; Table 2.2).

Aquatic prey taxa were detected in approximately 99% of nestling fecal samples with aquatic dipterans in the families Limoniidae (37%), Tabanidae (51%), and Tipulidae (60%) being among the most frequently detected taxa across nestling diets (Figure 2.2). Louisiana Waterthrush nestlings also consumed aquatic taxa in the orders of Decapoda (56%), Ephemeroptera (100%), Megaloptera (62%), Plecoptera (87%), and Trichoptera (28%), all of which were either rare or absent in the diets of Acadian Flycatcher or Wood Thrush nestlings (Table 2.2; Figure 2.2). The mean proportions (logit-transformed) of dietary taxa with aquatic stages differed significantly across nestling species ($X^2_{3,5} = 82.53$; $P < 0.001$; Figure 2.3). Pairwise comparisons revealed that Louisiana Waterthrush nestling diets were composed of a

TABLE 2.1. Percent frequency of occurrence of identified prey taxa in the diets of Acadian Flycatcher, Louisiana Waterthrush, and Wood Thrush nestlings. Shading indicates dietary taxa with an aquatic larval stage. Percent frequency of occurrence = number of fecal samples in which a taxon was detected divided by the total number of fecal samples (for each nestling species).

					% Frequency of Occurrence		
Class	**Order**	**Family**	**Genus**	**Species**	**Acadian Flycatcher (n = 44)**	**Louisiana Waterthrush (n = 39)**	**Wood Thrush (n = 51)**
Arachnida	Araneae	Anyphaenidae	*Anyphaena*	*pectorosa*	45.5		7.8
		Araneidae	*Neoscona*	*crucifera*	61.4		31.4
		Linyphiidae	*Pityohyphantes*	sp.	9.1	2.6	9.8
		Salticidae	*Naphrys*	*pulex*	15.9		7.8
			Pelegrina	*galathea*	18.2		5.9
		Tetragnathidae	*Leucauge*	*venusta*	9.1	2.6	17.6
		Theridiidae	*Parasteatoda*	*tepidariorum*	75.0		58.8
Diplopoda	Polydesmida	Paradoxosomatidae	*Oxidus*	*gracilis*			13.7
Insecta	Blattodea	Cryptocercidae	*Cryptocercus*	*punctulatus*	2.3	5.1	11.8
	Coleoptera	Carabidae	*Amphasia*	*interstitialis*	100.0		94.1
			Chlaenius	*impunctifrons*	9.1		19.6
			Platynus	sp.	11.4		7.8
			Sphaeroderus	*stenostomus*	6.8		19.6
		Cerambycidae	*Xestoleptura*	*octonotata*	6.8		13.7
		Elateridae	*Athous*	*brightwelli*	25.0		25.5
			Denticollis	*denticornis*	25.0		41.2
		Tenebrionidae	*Isomira*	*sericea*	9.1		9.8
	Diptera	Anthomyiidae	*Delia*	*platura*	6.8		7.8
		Asilidae	*Laphria*	sp.	20.5		5.9
				winnemana	15.9		19.6
		Chloropidae	*Tricimba*	sp.	11.4		25.5
		Limoniidae	*Austrolimnophila*	*toxoneura*	18.2		25.5
			Eutonia	*alleni*		23.1	
			Limnophila	*rufibasis*		20.5	2.0
			Limonia	*indigena*	4.5	10.3	13.7
			Rhipidia	*maculata*	2.3	15.4	2.0
		Muscidae	*Helina*	*evecta*	6.8		9.8
		Mycetophilidae	*Mycetophila*	*fungorum*	4.5		21.6
		Psychodidae	*Clogmia*	*albipunctata*	6.8		7.8
		Rhagionidae	*Rhagio*	*vertebratus*	36.4		13.7
		Sciaridae	*Schwenckfeldina*	*quadrispinosa*		7.7	9.8
		Syrphidae	*Myolepta*	*nigra*	36.4		13.7
			Syrphus	*knabi*	20.5		23.5
				rectus	34.1		15.7
			Xylota	sp.	31.8	10.3	2.0
		Tabanidae	*Goniops*	*chrysocoma*	34.1		29.4
			Hybomitra	sp.	6.8	17.9	2.0
			Tabanus	*milleri*	29.5		5.9
				sp.	54.5	15.4	51.0
		Tachinidae	*Blepharomyia*	*tibialis*	2.3	10.3	7.8
			Ceromya	*oriens*	9.1		23.5
			Lespesia	sp.	11.4		13.7
			Tachinomyia	*nigricans*	13.6		3.9
		Tipulidae	*Ctenophora*	*dorsalis*	31.8	7.7	35.3
			Leptotarsus	*testaceus*	22.7		9.8
			Tipula	*hermannia*	6.8	66.7	11.8
				longiventris		12.8	3.9
				oropezoides		20.5	
				sp.	22.7	41.0	43.1
		Xylomyidae	*Xylomya*	*pallidifemur*	29.5		43.1
	Ephemeroptera	Ameletidae	*Ameletus*	*lineatus*	4.5	17.9	9.8
		Ephemerellidae	*Ephemerella*	*dorothea*		48.7	
		Ephemeridae	*Ephemera*	*guttulata*		35.9	
		Heptageniidae	*Cinygmula*	*subaequalis*	6.8	33.3	2.0
			Epeorus	*pleuralis*	2.3	33.3	3.9
			Maccaffertium	*pudicum*	4.5	23.1	2.0
		Isonychiidae	*Isonychia*	sp.	11.4	35.9	5.9
		Leptophlebiidae	*Paraleptophlebia*	*guttata*	6.8		7.8

TABLE 2.1. *Continued.*

Class	Order	Family	Genus	Species	% Frequency of Occurrence: Acadian Flycatcher (n = 44)	Louisiana Waterthrush (n = 39)	Wood Thrush (n = 51)
Insecta	Hemiptera	Reduviidae	*Zelus*	*luridus*	9.1	2.6	5.9
	Lepidoptera	Erebidae	*Allotria*	*elonympha*	11.4		27.5
			Eulepidotis	*caeruleilinea*	6.8	7.7	25.5
			Hypena	*baltimoralis*	15.9	7.7	23.5
				edictalis	11.4	5.1	5.9
				scabra	9.1	5.1	2.0
			Hyperstrotia	*pervertens*	38.6		25.5
			Lophocampa	*maculata*	9.1		15.7
			Orgyia	*definita*	15.9		27.5
			Zale	*duplicata*	9.1		17.6
			Zanclognatha	*laevigata*	13.6	2.6	17.6
		Geometridae	*Ectropis*	*crepuscularia*	2.3	15.4	5.9
			Epimecis	*hortaria*	20.5	2.6	13.7
			Eupithecia	*columbiata*	6.8	10.3	17.6
			Lambdina	sp.	9.1		9.8
			Lomographa	*semiclarata*	18.2		5.9
			Melanolophia	*canadaria*	68.2		68.6
			Metarranthis	*hypochraria*	22.7		2.0
			Plataea	*calcaria*	6.8		9.8
			Probole	sp.	6.8		9.8
			Pseudasellodes	*fenestraria*	2.3		11.8
			Speranza	*pustularia*	9.1	7.7	3.9
		Gracillariidae	*Parornix*	*anglicella*	2.3		11.8
		Hesperiidae	*Ochlodes*	*sylvanus*	13.6	7.7	17.6
		Lasiocampidae	*Malacosoma*	*americanum*	31.8		39.2
				disstria	40.9		45.1
		Lycaenidae	*Parrhasius*	*album*	18.2		3.9
		Noctuidae	*Achatia*	*distincta*	29.5		27.5
			Anathix	*ralla*		15.4	2.0
			Eupsilia	sp.		10.3	7.8
			Hypotrix	*carnetincta*	22.7	2.6	11.8
			Lithophane	sp.	54.5	41.0	58.8
			Morrisonia	*confusa*	4.5	12.8	7.8
				latex	95.5	71.8	96.1
			Mythimna	*unipuncta*	18.2		13.7
			Ochropleura	*astigmata*	4.5	10.3	27.5
			Orthosia	*hibisci*		17.9	
				rubescens	36.4	46.2	56.9
			Protoschinia	*scutosa*	11.4		3.9
			Sunira	*bicolorago*	22.7		31.4
			Xestia	*nigrum*	4.5	2.6	9.8
		Nymphalidae	*Calisto*	*aquilum*	38.6		33.3
				sp.	11.4		5.9
		Pyralidae	*Plodia*	*interpunctella*	15.9		23.5
		Tortricidae	*Acleris*	*nigrolinea*	11.4		17.6
			Pseudexentera	*costomaculana*	43.2		9.8
				sp.		30.8	
		Zygaenidae	*Acoloithus*	*falsarius*	6.8		9.8
	Mecoptera	Panorpidae	*Panorpa*	sp.	13.6		13.7
	Megaloptera	Corydalidae	*Nigronia*	*fasciatus*	20.5	33.3	11.8
				serricornis	2.3	30.8	2.0
		Sialidae	*Sialis*	*joppa*		25.6	
	Orthoptera	Rhaphidophoridae	*Euhadenoecus*	*puteanus*	4.5	15.4	3.9
	Plecoptera	Leuctridae	*Leuctra*	*sibleyi*	4.5	5.1	11.8
				sp.		33.3	
		Perlidae	*Acroneuria*	*carolinensis*		71.8	
		Perlodidae	*Clioperla*	*clio*		23.1	
			Isoperla	sp.		25.6	
		Pteronarcyidae	*Pteronarcys*	*proteus*		38.5	
	Psocodea	Caeciliusidae	*Valenzuela*	*flavidus*	4.5	5.1	5.9
	Trichoptera	Goeridae	*Goera*	*stylata*		28.2	2.0
Malacostraca	Decapoda	Cambaridae	*Cambarus*	*carinirostris*		56.4	

TABLE 2.2. Percent frequency of occurrence of identified prey orders in the diets of Acadian Flycatcher, Louisiana Waterthrush, and Wood Thrush nestlings. Percent frequency of occurrence = number of fecal samples in which an order was detected divided by the total number of fecal samples (for each nestling species).

	% Frequency of Occurrence			
Order	**All spp. (n = 134)**	**Acadian Flycatcher (n = 44)**	**Louisiana Waterthrush (n = 39)**	**Wood Thrush (n = 51)**
Lepidoptera	99	100	97	100
Diptera	95	100	82	100
Coleoptera	70	100	0	98
Araneae	60	93	5	73
Ephemeroptera	51	34	100	27
Plecoptera	31	5	87	12
Megaloptera	30	23	62	12
Decapoda	16	0	56	0
Mecoptera	10	14	0	14
Trichoptera	9	0	28	2
Orthoptera	7	5	15	4
Blattodea	7	2	5	12
Hemiptera	6	9	3	6
Polydesmida	5	0	0	14
Psocodea	5	5	5	6

significantly larger proportion of aquatic prey taxa (0.69 ± 0.02 SE) compared to Acadian Flycatcher (0.17 ± 0.01 SE; $X^2_{3,4} = 58.26$; $P < 0.001$) and Wood Thrush nestlings (0.15 ± 0.01 SE; $X^2_{3,4} = 55.79$; $P < 0.001$; Figure 2.3). The proportion of taxa with aquatic stages in the diets of Acadian Flycatcher and Wood Thrush nestlings did not differ significantly ($X^2_{3,4} = 1.52$; $P = 0.218$; Figure 2.3).

Analysis of dietary niche overlap using Pianka's Index indicated that nestling species exhibited significantly more overlap than predicted by random simulations (Pianka 0.475; *P* =

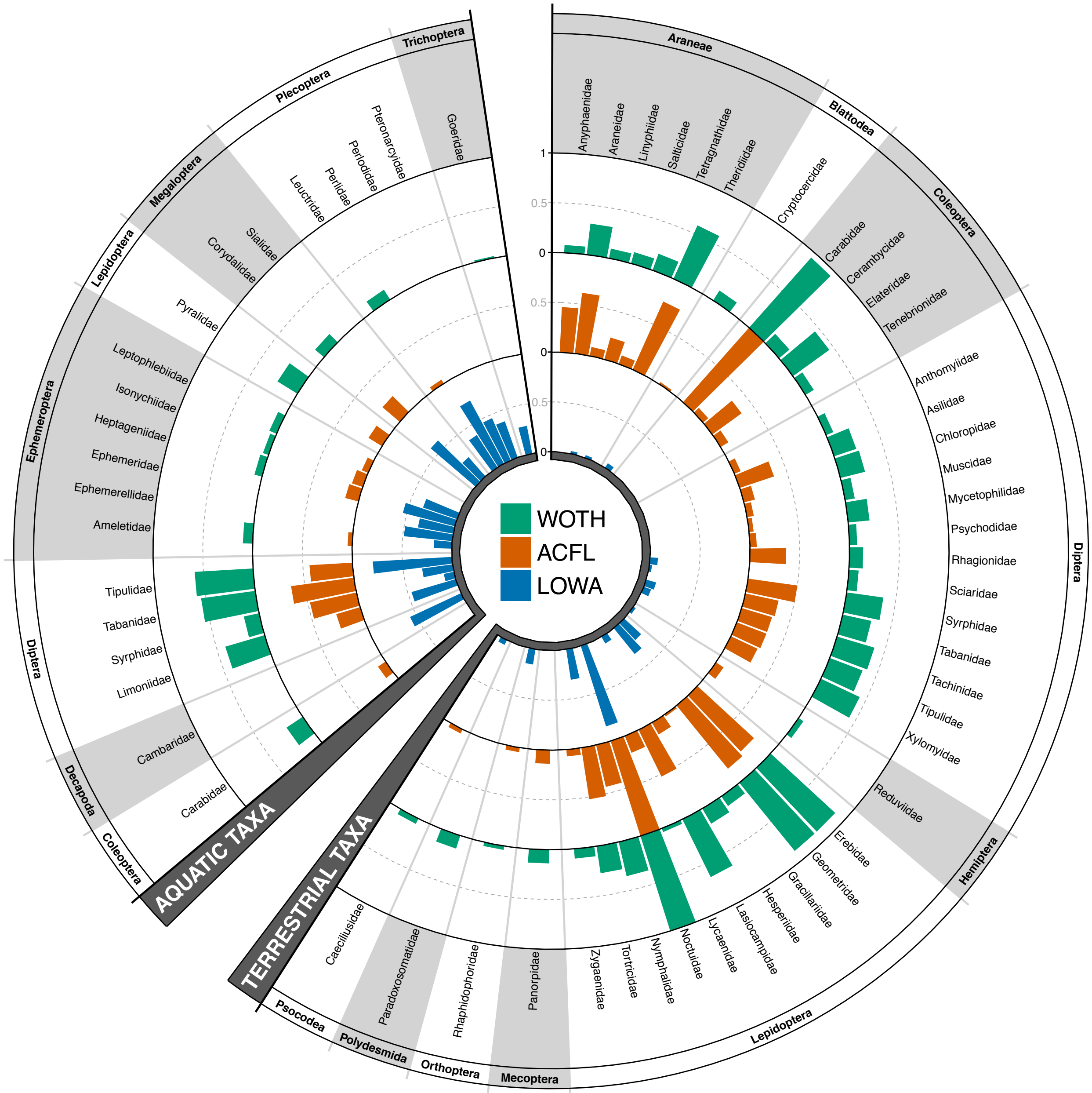

FIGURE 2.2. Frequency of occurrence of identified prey taxa (summarized by family) with an aquatic (left) or terrestrial (right) larval stage in the diets of Acadian Flycatcher (ACFL), Louisiana Waterthrush (LOWA), and Wood Thrush (WOTH) nestlings. Frequency of occurrence = number of fecal samples in which a taxon was detected divided by the total number of fecal samples (for each nestling species).

0.0001; Figure 2.4). Pairwise comparisons revealed a significant difference in observed and expected dietary overlap between Acadian Flycatcher and Wood Thrush nestlings (Pianka 0.844, P = 0.0001; Figure 2.4). In contrast, the diets of Acadian Flycatcher and Louisiana Waterthrush nestlings exhibited significantly less dietary niche overlap than expected (Pianka 0.249, P =

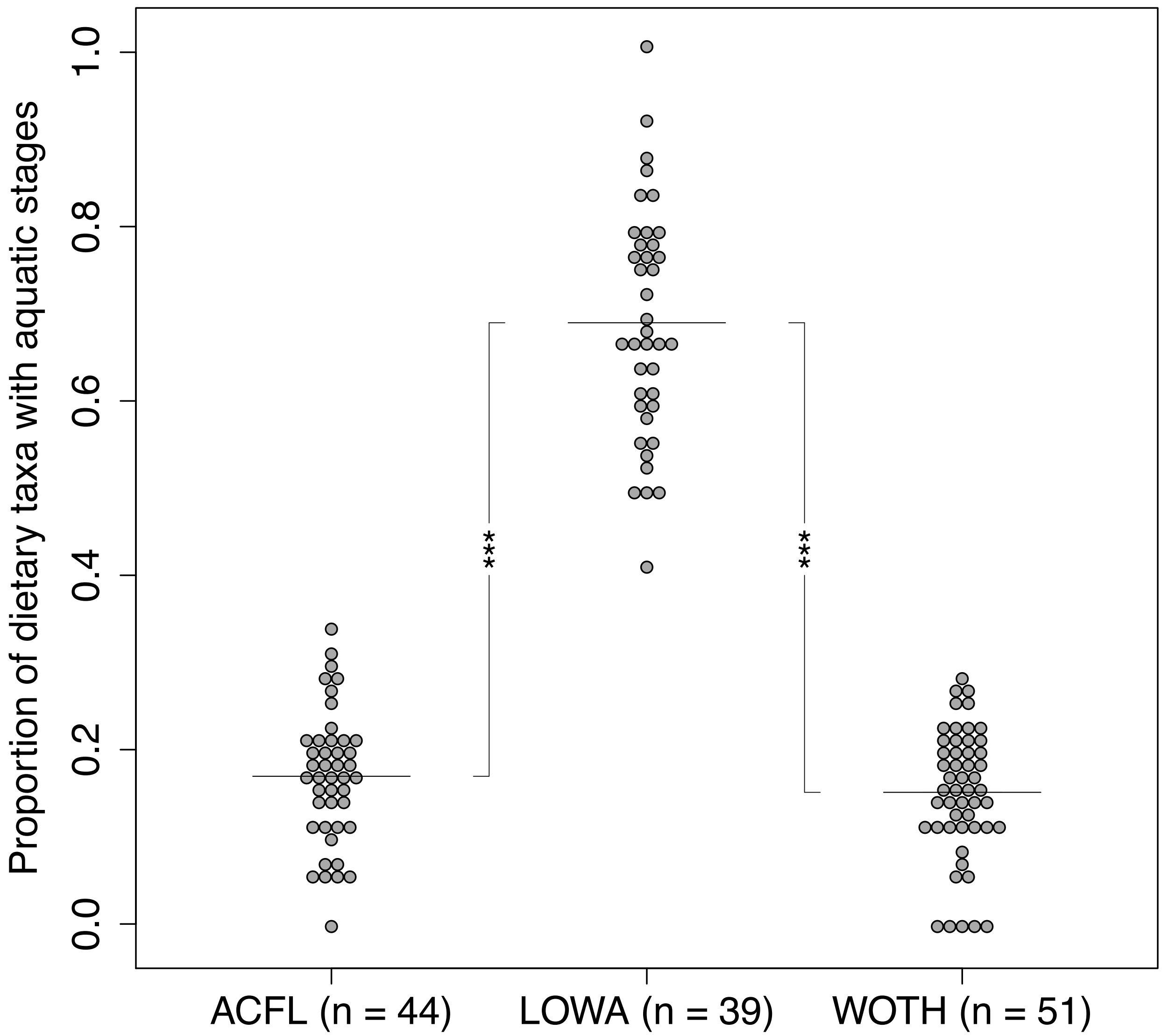

FIGURE 2.3. Proportions of identified prey taxa with aquatic larval stages in the diets of Acadian Flycatcher (ACFL), Louisiana Waterthrush (LOWA), and Wood Thrush (WOTH) nestlings. Points represent the proportion of dietary taxa with an aquatic larval stage in each nestling fecal sample and lines indicate mean proportions for each nestling species. Asterisks indicate a statistical difference between means (ANOVA; *** $P < 0.001$).

0.042). Dietary niche overlap between Louisiana Waterthrush and Wood Thrush nestlings did not differ significantly from the mean of random simulations (Pianka 0.331, $P = 0.465$).

The taxonomic composition of nestling diets (summarized by nest) differed significantly across focal species (PERMANOVA $Pseudo\text{-}F_{2,40} = 4.93$, $P = 0.001$) and in all pairwise models; however, the magnitude of dietary niche divergence was greatest between the nestlings of Louisiana Waterthrush and those of Acadian Flycatcher (PERMANOVA $Pseudo\text{-}F_{1,24} = 5.74$, SES = 17.03, $P = 0.001$) and Wood Thrush (PERMANOVA $Pseudo\text{-}F_{1,24} = 6.61$, SES = 16.74, $P = 0.001$). In contrast, dietary niche divergence between Acadian Flycatcher and Wood Thrush nestlings was less pronounced but still highly significant (PERMANOVA $Pseudo\text{-}F_{1,32} = 1.80$, SES = 3.85, $P = 0.003$). These results were reflected by unconstrained NMDS ordination, which generated a stable two-dimensional representation (stress = 0.216) of multivariate diet space that illustrated a significant divergence of Louisiana Waterthrush nestling diet relative to the other focal species (Figure 2.5). Furthermore, NMDS confirmed subtle but significant differences between the diets of Acadian Flycatcher and Wood Thrush nestlings (non-overlapping 95% CI ellipses for species centroids; Figure 2.5). Differences in nestling diet variability (multivariate homogeneity of dispersion) were marginally significant across all focal species (ANOVA $F_{2,40} = 2.69$, $P = 0.065$); however, pairwise comparisons revealed no statistical difference in diet variability between species ($P > 0.05$).

2.4 DISCUSSION

To our knowledge, this study represents the first application of DNA metabarcoding to study the diets among syntopic Neotropical migratory songbirds. This approach resulted in a genus- or species-level description of diet that revealed the consumption of aquatic taxa by nearly every nestling in this study (including songbirds generally considered terrestrial foragers). In fact, aquatic crane flies (Diptera: Limoniidae and Tipulidae) and horse flies (Diptera: Tabanidae) were among the most frequently consumed prey taxa across nestling diets (Figure 2.2) despite substantial differences in adult foraging strategies, supporting our hypothesis that aquatic subsidies are an important food resource for the

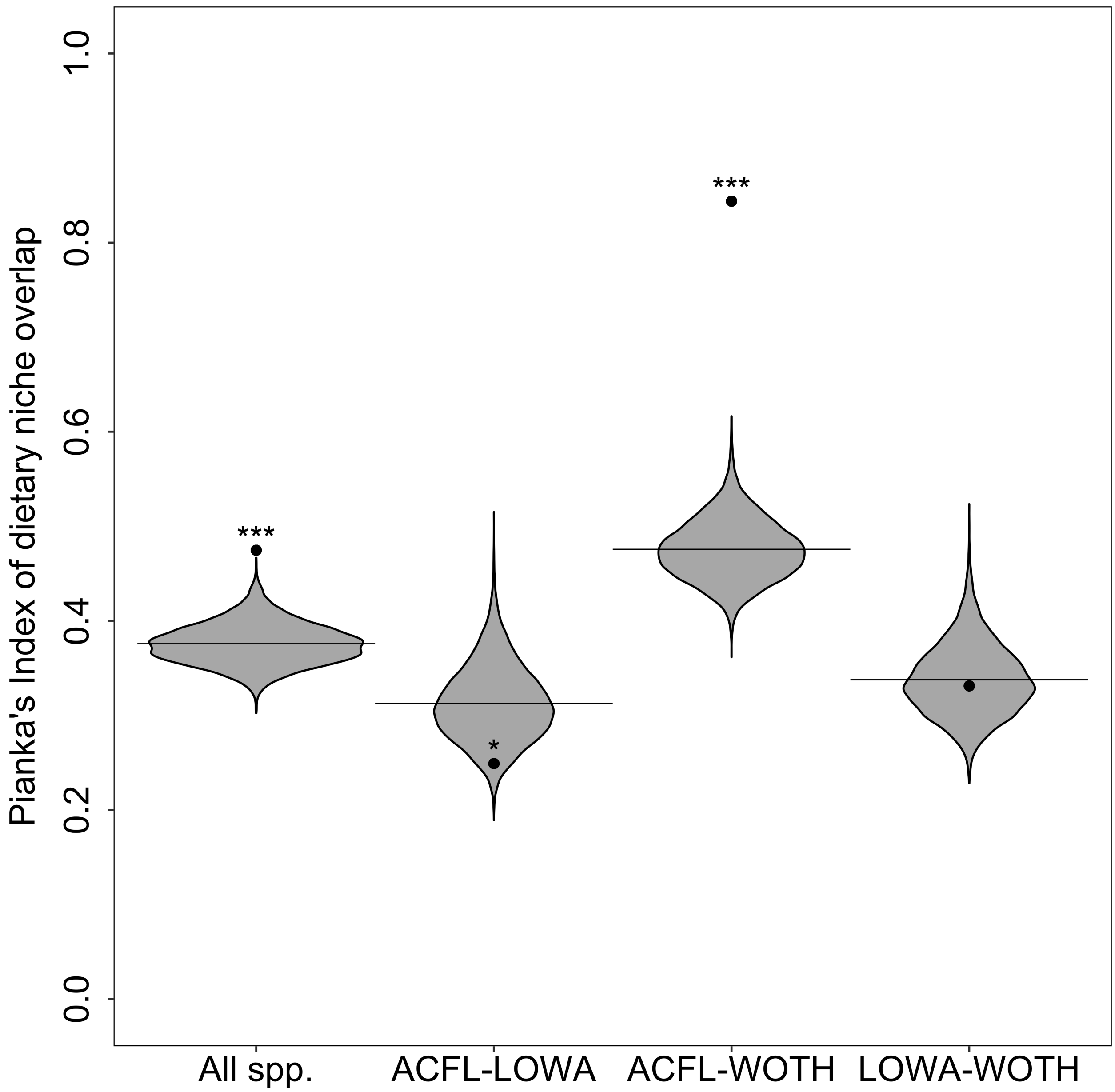

FIGURE 2.4. Pianka's Index of dietary niche overlap between Acadian Flycatcher (ACFL), Louisiana Waterthrush (LOWA), and Wood Thrush (WOTH) nestlings. Gray density plots represent Pianka's Index values generated from randomized MOTU frequency of occurrence data and lines indicate the mean overlap of 10,000 simulations (null hypothesis). Points represent observed dietary niche overlap between nestling species and asterisks indicate a significant difference between observed and expected diet overlap (****P* < 0.001; **P* < 0.05).

nestlings of Neotropical migratory songbirds in riparian habitats. These results are consistent with previous studies demonstrating that Neotropical migrants breeding in riparian habitats opportunistically prey upon emergent aquatic insects (e.g., Busby & Sealy 1979; Wiesenborn & Heydon 2007) and may preferentially target aquatic Diptera over larger prey taxa (e.g., Plecoptera) due to increased foraging efficiency and digestibility (e.g., Biermann & Sealy 1982; Raley & Anderson 1990). This preference for emergent aquatic Diptera has been shown to decrease the number of foraging trips for brooding females in riparian areas (Biermann & Sealy 1982), thereby increasing the probability of reproductive success by limiting the exposure of nestlings to brood parasites (Arcese & Smith 1988) and predators (Martin *et al.* 2000).

In addition to aquatic Diptera, Louisiana Waterthrush nestlings frequently consumed aquatic taxa in the orders Decapoda (crayfish), Ephemeroptera (mayflies), Megaloptera (dobsonflies and fishflies), Plecoptera (stoneflies), and Trichoptera (caddisflies). Despite being abundant throughout the nesting period of each focal species (B. Trevelline, unpublished data), aquatic taxa in these orders were either rare or completely absent from the diets of Acadian Flycatcher and Wood Thrush nestlings (Table 2.2). The prevalence of these aquatic prey taxa in Louisiana Waterthrush nestling diets was concomitant with the infrequent detection of terrestrial taxa that were common in Acadian Flycatcher and Wood Thrush nestling diets, resulting in a relatively narrow dietary niche (as determined by Levins' Index and a multivariate homogeneity of dispersion test) that differed significantly from other species of riparian nestlings (Figure 2.5). While it is possible that temporal differences in nesting periods may contribute to interspecific dietary niche divergence (Louisiana Waterthrush nesting peaks ~ 1 month earlier than Acadian Flycatcher), our results are consistent with the primarily aquatic foraging strategy employed by Louisiana Waterthrush and supports previous studies suggesting that this species is dependent on the availability benthic and emergent aquatic taxa (e.g., Mulvihill *et al.* 2008; Wood *et al.* 2016). Despite their specialized aquatic foraging strategy, 97% of Louisiana Waterthrush nestlings in this study consumed terrestrial Lepidoptera (Table 2.2; Figure 2.2), but primarily targeted families that are among the most abundant in Appalachian forests

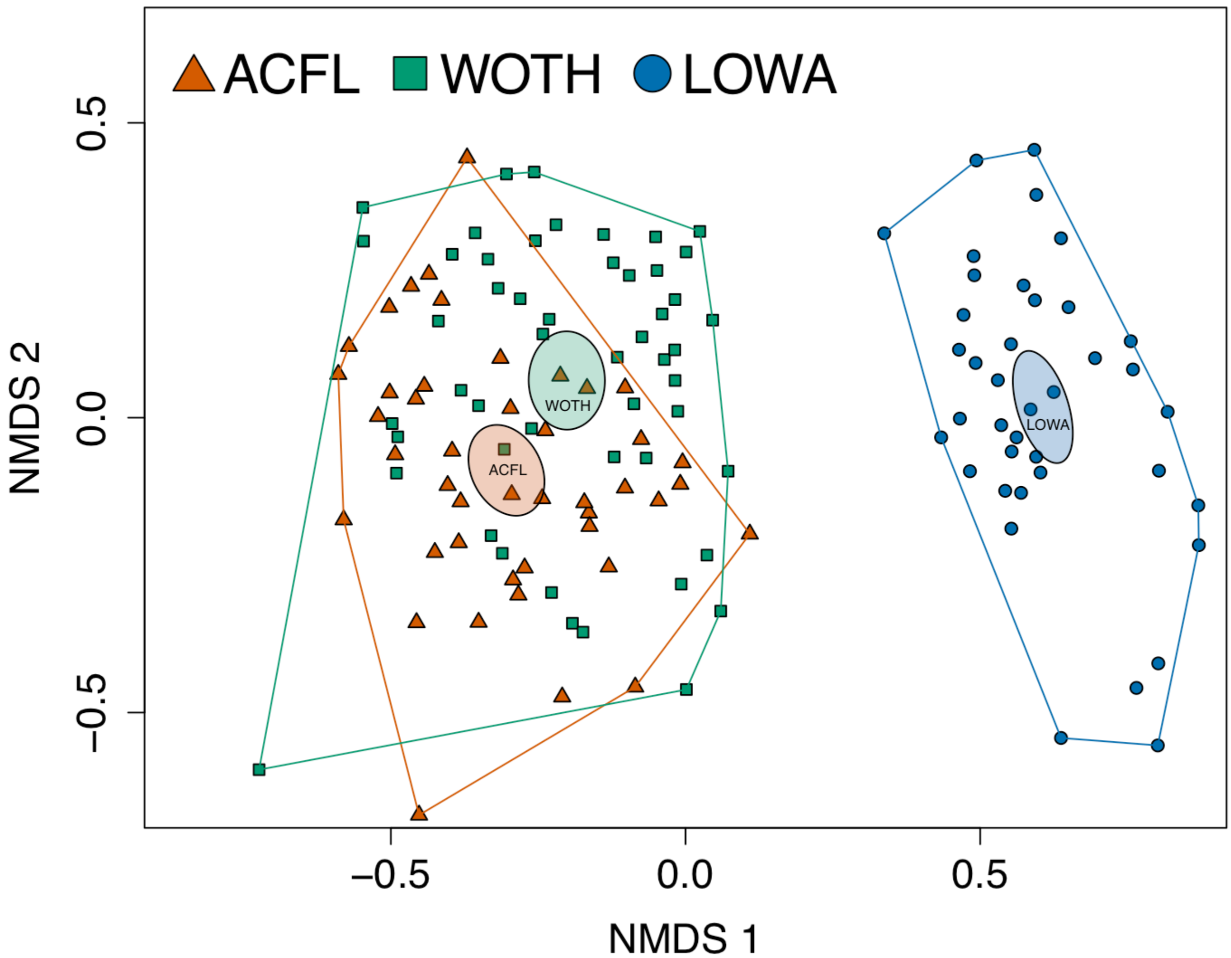

FIGURE 2.5. Unconstrained NMDS ordination of Acadian Flycatcher (ACFL), Louisiana Waterthrush (LOWA), and Wood Thrush (WOTH) nestling diet composition at the MOTU level. Points represent the taxonomic composition of each nestling diet, ellipses represent 95% confidence intervals for species centroids, and minimum convex polygons indicate the extent of dietary niche space for each species.

(Geometridae and Noctudiae; Wheatall *et al.* 2013). These results are consistent with recent evidence that Louisiana Waterthrush may deviate from their typical aquatic foraging strategy to opportunistically provision Lepidoptera (Trevelline *et al.* 2016), which are high-quality prey frequently targeted by other species of Neotropical migrants during the period of nestling care (e.g., Holmes *et al.* 1979b).

While our results provide support for the hypothesis that breeding Neotropical migrants occupy distinct dietary niches that reflect divergent foraging strategies (Figure 2.5), we reported significant community-level overlap that was primarily driven by a high degree of dietary similarity between Acadian Flycatcher and Wood Thrush nestlings (Figure 2.4). Given that Neotropical migrants with divergent foraging strategies typically exhibit substantial differences in diet (e.g., MacArthur 1958; Strong 2000; this study), the observed dietary overlap between Acadian Flycatcher and Wood Thrush nestlings was unexpected. In accordance with resource partitioning theory (*sensu* Cody 1968), we predicted that these syntopic insectivores would consume different prey taxa in order to minimize interspecific competition over access to limited food resources. During peak periods of insect availability, however, the diets of breeding birds with drastically different foraging strategies and morphology have been shown to converge as a result of the opportunistic consumption of abundant prey taxa (Rosenberg *et al.* 1982; Rotenberry 1980; Wiens & Rotenberry 1979). Therefore, we contend that the observed dietary overlap between Acadian Flycatcher and Wood Thrush nestlings should not be interpreted as competition between species with different foraging strategies, but rather as evidence for dietary opportunism permitted by an abundance of insect prey in riparian habitats that occurred while both species were nesting (as opposed to the earlier nesting cycle exhibited by Louisiana Waterthrush). While this interpretation is consistent with the competing theory that food resources are generally non-limiting throughout the North American breeding grounds (Rappole & McDonald 1994; Wiens 1977), we suggest a more tentative conclusion considering that prey availability in riparian areas is known to be exceptionally high compared to other habitat types (Baxter *et al.* 2005).

Our description of Neotropical migrant nestling diets used data from a single breeding season along nearby streams. Considering that the diets of breeding songbirds can vary drastically between locations and years (e.g., Rotenberry 1980; Wiens & Rotenberry 1979), the taxonomic composition of diets presented here should not be considered representative descriptions. For example, 56% of Louisiana Waterthrush nestlings in this study consumed crayfish (family Cambaridae; Figure 2.2), but crayfish were not detected in any nestling diets in our previous study conducted in 2013 (Trevelline *et al.* 2016). Similarly, Trevelline *et al.* (2016) demonstrated that 7% of Louisiana Waterthrush nestlings consumed Plecoptera compared to 87% in the present study (Table 2.2). While Wiens and Rotenberry (1979) reported similar degrees of inter-annual variation in diets, our case is especially notable considering that these studies were conducted in the same breeding territories just two years apart. For our study sites, inter-annual variation in Louisiana Waterthrush nestling diet composition may be partially explained by a form of aquatic insect development that requires several years to reach maturity and emerge (common in some Plecoptera; Merritt & Cummins 2008), thus emphasizing the importance of dietary plasticity in Neotropical migrants that rely on the availability of ephemeral prey resources.

Our molecular approach utilized a single COI primer set designed for the detection of arthropod prey (Zeale *et al.* 2011); therefore, our description of nestling diets should not be considered comprehensive. Ideally, our methodology would include multiple primer sets or additional barcoding genes, which may result in the detection of a greater variety of prey taxa (e.g., Bowser *et al.* 2013). This limitation may be particularly relevant to analyses of interspecific dietary niche overlap involving Wood Thrush, which occasionally feed nestlings non-arthropod prey (e.g., salamanders; Evans *et al.* 2011) that would not be detected using the present approach. Furthermore, molecular diet analyses are incapable of estimating the abundance of dietary prey items due to differences in prey size, digestion rates, and PCR amplification biases (Pompanon *et al.* 2012). Together, these limitations could potentially exaggerate dietary niche overlap, but should not diminish the conclusion that aquatic taxa are a major component of nestling diets across several species of Neotropical migrants with substantially different foraging strategies.

In this study, we generated a genus- or species-level description of nestling diets that refined our understanding of how aquatic prey subsidies support Neotropical migrants nesting in riparian ecosystems. For example, previous studies have suggested that emergent aquatic Diptera are important prey for the nestlings of Neotropical migrants breeding in riparian habitats (e.g., Biermann & Sealy 1982), but these studies were primarily restricted to order-level identifications due to the limitations of traditional morphological diet analyses (Rosenberg & Cooper 1990). In contrast, our approach revealed the consumption of 26 species (in 27 genera) of aquatic dipterans (Table 2.2), many of which are soft-bodied and may have escaped detection using traditional techniques (Rosenberg & Cooper 1990). This improved understanding of riparian nestling diets may be especially valuable for the conservation of the aquatic specialist Louisiana Waterthrush, which have been shown to respond negatively to the reduced availability of taxa in the order Ephemeroptera (Mulvihill *et al.* 2008). While our results provide support for the hypothesis that Ephemeroptera are important prey for Louisiana Waterthrush nestlings (Table 2.2), we further refined this broad understanding of diet by revealing the frequent consumption of genera that are highly sensitive to disturbances in water quality (e.g., *Ephemerella;* 49%; Table 2.1; Barbour *et al.* 1999) and others that emerge every other year (e.g., *Ephemera;* 36%; Table 2.1; Merritt & Cummins 2008). Because pollution-sensitive aquatic taxa are often reduced or absent in catchments disturbed by anthropogenic activities (e.g., Mulvihill *et al.* 2008; Wood *et al.* 2016), our results suggest that riparian habitat degradation may negatively impact the breeding productivity of Neotropical migratory songbirds, and thus the long-term conservation of avian diversity in riparian ecosystems.

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CHAPTER THREE

Stream acidification and reduced availability of pollution-sensitive aquatic insects alter the diet of a stream-dependent Neotropical migratory songbird

Headwater mountain streams and the riparian forests that surround them are inextricably linked by reciprocal exchanges of prey essential to both aquatic and terrestrial predators. Aquatic arthropods comprise a large proportion of total prey availability in riparian habitats and are opportunistically exploited by terrestrial insectivores; however, the use of aquatic prey resources is obligatory for several species of songbirds that utilize specialized aquatic foraging strategies. For these stream-dependent songbirds, reduced availability of pollution-sensitive aquatic taxa is associated with negative impacts to nestling physiology and survival, which are typically accompanied by compensatory changes in foraging behavior that may result in substantial dietary shifts. We utilized DNA metabarcoding to investigate potential dietary shifts in response to stream pH and the availability of pollution-sensitive aquatic prey in a stream-dependent Neotropical migratory songbird, the Louisiana Waterthrush (*Parkesia motacilla*). Our results revealed that both adult and nestling waterthrush occupying territories with reduced pH and availability of pollution-sensitive aquatic taxa exhibited significant dietary shifts compared to conspecifics in higher quality territories. These shifts were primarily driven by an expansion of prey taxa and overall dietary niche breadth resulting from the consumption of terrestrial prey. This relationship between stream quality and diet was not observed for other syntopic species of Neotropical migrants nesting in the same riparian habitat. In addition to providing support for our hypothesis that Louisiana Waterthrush compensate for food limitations by targeting terrestrial arthropods in degraded riparian habitats, our findings emphasize the vulnerability of Louisiana Waterthrush to anthropogenic disturbances that compromise stream quality or reduce the availability of pollution-sensitive aquatic insects.

3.1 INTRODUCTION

Headwater mountain streams and the riparian forests that surround them are inextricably linked by reciprocal exchanges of prey essential to both aquatic and terrestrial consumers (Baxter *et al.* 2005; Polis *et al.* 1997). Arthropods with aquatic larval stages comprise a large proportion of total prey availability in riparian habitats (Nakano & Murakami 2001) and are opportunistically exploited by terrestrial consumers (e.g., Gray 1993; Rosenberg *et al.* 1982; but see Chapter 2), often resulting in a more diverse and densely populated assemblage of insectivores compared to adjacent non-riparian habitats (reviewed in Baxter *et al.* 2005). For several species, however, the use of aquatic prey resources is obligatory (e.g., Krüger *et al.* 2014; Mattsson *et al.* 2009; Wilson & Kingery 2011), and thus these stream-dependent terrestrial insectivores may be vulnerable to land-use changes that disrupt the availability of aquatic invertebrates.

The availability of aquatic arthropods as prey for stream-dependent songbirds is largely determined by both chemical and geomorphic factors that are strongly influenced by anthropogenic activities (Rosenberg & Resh 1993). For example, anthropogenic disturbances to riparian habitats such as abandoned mine discharge (Tomkiewicz & Dunson 1977), acid precipitation (Graveland 1998), hydraulic fracture (Wood *et al.* 2016), thermal pollution (Benke 1993), and urbanization (Roy *et al.* 2003) have been shown to alter the composition of riparian insect communities primarily through the reduced availability of pollution-sensitive aquatic taxa (particularly those in the orders Ephemeroptera, Plecoptera, and Trichoptera; hereafter EPT).

Riparian zones support several species of songbirds that are thought to specialize on pollution-sensitive EPT taxa (e.g., Mattsson *et al.* 2009; Ormerod & Tyler 1991; Wilson & Kingery 2011), and thus riparian habitats with reduced availability of these prey items support fewer breeding stream-dependent species compared to unimpacted drainages (Buckton *et al.* 1998; Feck & Hall 2004; Mulvihill *et al.* 2008; Ormerod *et al.* 1986). Nevertheless, poor-quality riparian territories often remain occupied, typically by inexperienced breeding pairs (second-year birds; Mulvihill *et al.* 2008). Stream-dependent songbirds occupying acidified territories with reduced access to EPT prey often exhibit delayed clutch initiation (Mulvihill *et al.* 2008), smaller clutches (Ormerod *et al.* 1991), thinner egg shells (Ormerod *et al.* 1988), reduced nestling growth rate (Ormerod *et al.* 1991), lower nestling serum calcium levels (Ormerod *et al.* 1991), increased rates of nestling predation (O'Halloran *et al.* 1990), reduced nestling survival (Vickery 1992), fewer nesting attempts (Mulvihill *et al.* 2009), and lower reproductive success (Wilson & Kingery 2011). Because these factors are thought to influence the annual breeding productivity of stream-dependent songbirds (e.g., Mattsson & Cooper 2007) and migrants in general (reviewed in Martin 1987), the reduced availability of EPT prey due to stream acidification may threaten the long-term conservation of birds that breed in riparian habitats.

For stream-dependent songbirds occupying anthropogenically degraded riparian habitats, the observed negative impacts to reproduction and nestling survival are typically coupled with changes in foraging behavior. For example, Louisiana Waterthrush (*Parkesia motacilla*) nesting in acidified riparian habitats with reduced EPT availability expand their breeding territories and forage along unimpacted peripheral tributaries more frequently (Mulvihill *et al.* 2008). Similar behavioral responses have been observed in stream-dependent dippers (genus *Cinclus*), where individuals breeding in degraded habitats expand their foraging areas (Wilson & Kingery 2011), spend more time away from the nest (O'Halloran *et al.* 1990), and feed nestlings less frequently (Vickery 1992). These behavioral shifts are thought to be a compensatory response to the reduced availability of pollution-sensitive EPT taxa (Mulvihill *et al.* 2008; O'Halloran *et al.*

1990), which have been shown to be important prey during the period of nestling care (Mattsson *et al.* 2009; see Chapters 1 and 2). For other species of migratory songbirds, such shifts in foraging behavior are typically accompanied by a concomitant shift in diet (e.g., Cooper *et al.* 1990; Sample *et al.* 1993); however, it is unclear how the diets of stream-dependent songbirds are altered by stream acidification and reduced EPT availability.

In this study, we utilized DNA barcoding and next-generation sequencing (hereafter DNA metabarcoding) to investigate dietary shifts in a stream-dependent Neotropical migratory songbird, the Louisiana Waterthrush. We hypothesized that Louisiana Waterthrush occupying territories with reduced pH and EPT availability compensate by (1) expanding their dietary niche, and (2) targeting terrestrial arthropods.

3.2 MATERIALS AND METHODS

Study species and sample collection

The Louisiana Waterthrush is a riparian-obligate wood-warbler (family Parulidae) that nests directly in the banks of headwater mountain streams and primarily forages for aquatic insects (both larval and adult) in riffles and along stream edges (~ 90% of foraging maneuvers directed at water, but occasionally glean insects from foliage; Mattsson *et al.* 2009). Louisiana Waterthrush populations are declining throughout their range (Sauer *et al.* 2014) and is considered a species of conservation concern due to its dependence on high quality riparian areas and aquatic invertebrates that are sensitive to changes in water quality (Mattsson *et al.* 2009; Prosser & Brooks 1998).

We systematically located and monitored Louisiana Waterthrush nests within known breeding territories (consistently occupied each breeding season) along three headwater

Appalachian streams near Powdermill Nature Reserve (Westmoreland County, PA, USA) from April to June 2015: Laurel Run, Loyalhanna Creek, and Powdermill Run. We measured stream pH at a consistent location within each waterthrush breeding territory throughout the 2014 and 2015 breeding season using a handheld multi-parameter instrument (YSI Inc., Yellow Springs, OH, USA). These measurements were used to assess differences in waterthrush territory quality using a linear mixed-effects model with random terms to account for the clustering of territories along the same stream in the R package *lme4* (Bates *et al.* 2015; function: lmer). Furthermore, we used these measurements to calculate the mean pH of waterthrush territories over a two-year period, which were then used in subsequent linear and logistic regression models investigating dietary shifts.

To assess differences in prey availability between waterthrush territories, emergent EPT taxa were continuously collected (at pH monitoring locations) throughout the entire 2015 breeding season (April-June) using sticky traps (Olson Products Inc., Medina, OH; Collier & Smith 1995) and analyzed using a linear mixed-effects model with random terms to account for the clustering of territories along the same stream in the R package *lme4* (Bates *et al.* 2015; function: lmer). Because Louisiana Waterthrush are known to target both larval and emergent life-stages of aquatic arthropods (Mattsson *et al.* 2009), our characterization of EPT availability during the period of nestling care also included larval-stage benthic macroinvertebrates (collected using a D-frame dip net; Barbour *et al.* 1999). All EPT taxa collected via sticky traps and benthic sampling (300 individuals ± 20%; Barbour *et al.* 1999) were identified to family using the diagnostic morphological characteristics provided by Merritt and Cummins (2008). The availability of EPT taxa (the total number of EPT individuals in sticky traps and benthic samples collected within 1 week of egg hatching divided by the total number of individuals) for each waterthrush territory was used in subsequent linear and logistic regression models investigating dietary shifts. Like Mulvihill *et al.* (2008), we excluded the acid-tolerant families Leuctridae and Nemouridae (order Plecoptera) from our estimation of EPT availability in order to assess the impact of stream acidification on the diet of Louisiana Waterthrush.

Nestling fecal samples were collected by placing nestlings (4-8 days old) into a clean paper bag (for up to 3 minutes) or by encouraging voidance directly over an open 20 mL vial of 100% ethanol. When possible, nestling fecal samples were collected on a second occasion 1-2 days later. Adults associated with each nest were captured using targeted mist-netting and briefly (3-5 minutes) placed into a clean paper bag lined with a clean 1-quart plastic bag (left open) to facilitate collection of fecal material. Adult fecal material was transferred from plastic bags into a 20 mL vial using a sterile serological pipette and 100% ethanol. All fecal samples were stored -20°C for approximately 3 months prior to DNA extraction.

Molecular analysis and bioinformatics

Arthropod prey DNA was extracted using a protocol optimized for avian fecal samples (Appendix A; Trevelline *et al.* 2016) and amplified using polymerase chain reaction (PCR) and general arthropod primers designed to target a 157 bp region of the mitochondrial cytochrome c oxidase I barcoding gene (COI; Zeale *et al.* 2011). PCR amplification was performed in duplicate for each fecal sample (e.g., Crisol-Martínez *et al.* 2016; Trevelline *et al.* 2016; but see justification for triplicate PCR in Vo & Jedlicka 2014) and pooled for an additional indexing reaction using the Illumina Nextera XT (v2) Indexing Kit following the manufacturer's instructions (see Chapter 1; Trevelline *et al.* 2016). Once indexed, amplicon libraries were pooled at equimolar concentrations for analysis (250 bp paired-end) using the Illumina MiSeq next-generation sequencing platform.

Raw Illumina sequence reads were trimmed and quality filtered (Phred ≥ 30) using CLC Genomics Workbench 7.0.3 (Qiagen) and Galaxy 15.10 (Blankenberg *et al.* 2010; Giardine *et al.* 2005; Goecks *et al.* 2010). Remaining sequences were clustered into molecular operational taxonomic units (MOTUs) based on 97% similarity using QIIME 1.8.10 (Caporaso *et al.* 2010) and filtered to remove infrequent haplotypes (see details in Chapter 1.2; Trevelline *et al.* 2016). Representative sequences from each MOTU were queried in the Barcode of Life Database (BOLD; Ratnasingham & Hebert 2007) and scored based on taxonomic resolution and match to a reference sequence (Appendix B; Trevelline *et al.* 2016). To minimize the likelihood of taxonomic misidentifications from short fragments (157 bp) of the full-length (658 bp) COI barcoding region, MOTUs that exhibited < 98% similarity to a reference sequence or could not provide genus- or species-level resolution were classified as "unidentified" and excluded from taxonomic descriptions of diet (discussed in Clare *et al.* 2011). Because the proportion of sequencing reads does not necessarily reflect the relative quantities of prey consumed (Pompanon *et al.* 2012), the number of reads assigned to each dietary MOTU were transformed into a presence-absence dataset, which was used to calculate dietary MOTU frequency of occurrence (number of fecal samples in which a MOTU was detected divided by the total number of fecal samples) for both nestling and adult waterthrush.

Diet analysis

We determined the dietary richness of adult and nestling waterthrush based on the total number of MOTUs (including those that were unidentified; discussed in Clare *et al.* 2011) detected in fecal samples. We used the frequency of occurrence of dietary MOTUs among nestlings and adults (when possible) associated with the same nest to calculate total dietary niche breadth for each nest using Levins' Index (reciprocal of Simpson's Index of diversity; Levins 1968) in the R package *vegan* (Oksanen *et al.* 2017; function: diversity, index = "invsimpson"). Levins' Index of dietary niche breadth was standardized based on the total number of MOTUs in the diets of waterthrush to generate a value ranging from 0 to 1, where 1 represents a diet consisting of all detected MOTUs (Hurlbert 1978). Taxonomic dietary descriptions were summarized by frequency of occurrence at the order and MOTU level. Identified dietary MOTUs with an aquatic larval stage (hereafter "aquatic prey taxa") and those without an aquatic larval stage (hereafter "terrestrial prey taxa") were classified as such using the genus-level life history characteristics provided by Merritt and Cummins (2008).

To test the hypothesis that Louisiana Waterthrush shift their diets in response to disturbances in stream quality, changes in dietary MOTU richness and Levins' Index of niche breadth in response to stream pH and EPT availability were analyzed using linear mixed-effects models in the R package *lme4* (Bates *et al.* 2015; function: lmer). Linear mixed-effects models included random terms to account for the clustering of nests on the same stream and fecal samples associated with the same nest. The dietary niches of waterthrush occupying territories that differed in pH and EPT availability were visualized using non-metric multidimensional scaling (NMDS; Kruskal 1964) in *vegan* (Oksanen *et al.* 2017; function: metaMDS, distance = "jaccard", k = 2), which generates a two-dimensional unconstrained ordination plot that illustrates compositional differences between individual diets using minimum convex polygons (function: ordihull) and 95% confidence ellipses around species centroids (function: ordiellipse, kind = "se", conf = 0.95). To determine if waterthrush are particularly vulnerable to changes in stream quality due to aquatic foraging strategy, we applied these linear mixed-effects and NMDS models to investigate dietary shifts in the nestlings of two species of Neotropical migrants—Acadian Flycatcher (*Empidonax virescens*) and Wood Thrush (*Hylocichla mustelina*)—nesting in the same riparian habitat but primarily consume terrestrial arthropods (data from Chapter 2).

To test the hypothesis that Louisiana Waterthrush compensate for reduced EPT availability by targeting terrestrial arthropods, differences in terrestrial dietary MOTU richness (total number of identified taxa without an aquatic life-stage) in response reduced pH and perent EPT were analyzed using linear mixed-effects models in the R package *lme4* (Bates *et al.* 2015; function: lmer). Linear mixed-effects models included random terms to account for the clustering of nests on the same stream and fecal samples associated with the same nest. We determined whether the presence of identified dietary MOTUs was correlated with percent EPT using logistic regression in a generalized linear mixed-effects model (with the same random terms) in the R package *lme4* (Bates *et al.* 2015; function: glmer).

3.3 RESULTS

We successfully sequenced COI barcodes from 78 nestling (representing 10 nests) and 14 adult (breeding pairs from 7 of the 10 nests; Supplemental Tables C.3 and C.4, Appendix C) fecal samples collected from Louisiana Waterthrush occupying territories with significant differences in pH ($X^2_{3,12} = 75.16$; $P < 0.001$; Supplemental Tables C.5, Appendix C) and marginally significant differences in the availability of emergent EPT taxa ($X^2_{3,12} = 16.75$; $P = 0.053$; Supplemental Table C.6, Appendix C). After quality filtering and trimming, Illumina sequencing generated a total of 1,783,010 reads (mean of 19,381 per sample ± 10,454 SD) that clustered into 254 MOTUs after the removal of infrequent haplotypes (see Chapter 1.2 for details). Identification of MOTU representative sequences (Supplemental Data D.4, Appendix D) in the BOLD reference library resulted in ≥ 98% match to genus or species for 122 MOTUs (~ 48% of total MOTUs) representing 94 unique dietary taxa (Table 3.1).

Louisiana Waterthrush dietary richness ranged from 7 to 67 MOTUs (mean of 31.5 per sample ± 13.4 SD) and increased significantly as mean territory pH declined ($X^2_{5,6}$ = 10.80; P = 0.001; Figure 3.1, Panel A). This trend was observed for both adults ($X^2_{4,5}$ = 4.97; P = 0.026) and nestlings ($X^2_{5,6}$ = 11.72; $P < 0.001$). Like dietary MOTU richness, the total dietary niche breadth (nestlings and adults associated with the same nest) increased significantly as mean territory pH declined ($X^2_{4,5}$ = 4.05; P = 0.026; Figure 3.1, Panel B). NMDS analysis revealed that the diets of Louisiana Waterthrush occupying territories with reduced stream pH were distinct from conspecifics in more circumneutral territories (non-overlapping 95% CI ellipses around centroids; Figure 3.1, Panel C). In contrast, the dietary MOTU richness of Acadian Flycatcher ($X^2_{4,5}$ = 0.16; P = 0.69; n = 44; Figure 3.2) and Wood Thrush ($X^2_{4,5}$ = 1.14; P = 0.29; n = 51; Figure 3.3) nestlings in the same riparian habitat were unaffected by reduced EPT availability. Dietary MOTU richness increased significantly as percent EPT taxa declined ($X^2_{4,5}$ = 4.97; P = 0.026; Figure 3.4, Panel A). This trend was significant for both adults ($X^2_{4,5}$ = 5.52; P = 0.019) and nestlings ($X^2_{5,6}$ = 4.64; P = 0.031). In contrast, the dietary MOTU richness of Acadian Flycatcher ($X^2_{4,5}$ = 0.12; P = 0.73; Figure 3.5) and Wood Thrush ($X^2_{4,5}$ = 2.98; P = 0.084; Figure 3.6) nestlings was unaffected by reduced EPT availability. An increase in total dietary niche breadth of waterthrush nests in response to reduced EPT availability were marginally significant ($X^2_{4,5}$ = 3.62; P = 0.057; Figure 3.4, Panel B). NMDS analysis revealed that the diets of waterthrush in riparian habitats with reduced EPT availability were distinct from conspecifics with greater EPT availability (Figure 3.4, Panel C).

TABLE 3.1. Percent frequency of occurrence of identified arthropod MOTUs in the diets of adult and nestling Louisiana Waterthrush. Shading indicates dietary taxa with an aquatic larval stage. Percent frequency of occurrence = number of fecal samples in which a taxon was detected divided by the total number of adult and/or nestling fecal samples.

					% Frequency of Occurrence		
Class	**Order**	**Family**	**Genus**	**Species**	**Overall (n = 92)**	**LOWA Adults (n = 14)**	**LOWA Nestlings (n = 78)**
Arachnida	Araneae	Lycosidae	*Piratula*	*insularis*	5.4	7.1	5.1
		Philodromidae	*Philodromus*	*rufus*	5.4	7.1	5.1
	Trombidiformes	Protziidae	*Protzia*	*sp*	6.5	7.1	6.4
Insecta	Blattodea	Cryptocercidae	*Cryptocercus*	*punctulatus*	8.7		10.3
	Coleoptera	Curculionidae	*Sciaphilus*	*asperatus*	7.6		9.0
	Diptera	Chironomidae	*Krenopelopia*	sp.	51.1	42.9	52.6
		Culicidae	*Anopheles*	sp.	20.7	35.7	17.9
		Dolichopodidae	*Gymnopternus*	*spectabilis*	8.7	21.4	6.4
		Empididae	*Rhamphomyia*	sp.	10.9	7.1	11.5
		Limoniidae	*Austrolimnophila*	*toxoneura*	5.4	21.4	2.6
			Euphylidorea	*adustoides*	5.4	14.3	3.8
			Eutonia	*alleni*	21.7	14.3	23.1
			Limnophila	*rufibasis*	18.5	35.7	15.4
			Limonia	*indigena*	22.8	28.6	21.8
			Metalimnobia	*immatura*	10.9	7.1	11.5
			Rhipidia	*maculata*	14.1	14.3	14.1
		Pediciidae	*Pedicia*	sp.	10.9	14.3	10.3
			Tricyphona	*katahdin*	15.2	21.4	14.1
		Rhagionidae	*Symphoromyia*	*fulvipes*	6.5		7.7
		Sciaridae	*Schwenckfeldina*	*quadrispinosa*	5.4		6.4
		Stratiomyidae	*Allognosta*	*fuscitarsis*	7.6	14.3	6.4
		Syrphidae	*Somula*	*decora*	8.7	14.3	7.7
			Temnostoma	*alternans*	13.0	14.3	12.8
				sp.	5.4		6.4
			Xylota	*quadrimaculata*	6.5	14.3	5.1
		Tabanidae	*Chrysops*	sp.	5.4	21.4	2.6
			Hybomitra	*pechumani*	5.4		6.4
				sp.	28.3	35.7	26.9
			Tabanus	sp.	13.0	21.4	11.5
		Tachinidae	*Blepharomyia*	*tibialis*	14.1	14.3	14.1
			Compsilura	*concinnata*	10.9	14.3	10.3
		Tipulidae	*Ctenophora*	*dorsalis*	8.7	7.1	9.0
			Dolichopeza	*subvenosa*	14.1	14.3	14.1
			Tipula	*duplex*	5.4		6.4
				hermannia	66.3	92.9	61.5
				longiventris	14.1	21.4	12.8
				oropezoides	16.3	28.6	14.1
				sp.	48.9	57.1	47.4
	Ephemeroptera	Ameletidae	*Ameletus*	*lineatus*	34.8	50.0	32.1
				sp.	7.6	7.1	7.7
		Baetidae	*Baetis*	*phoebus*	7.6	14.3	6.4
				sp.	7.6	14.3	6.4
		Ephemerellidae	*Ephemerella*	*dorothea*	47.8	57.1	46.2
			Eurylophella	*funeralis*	7.6	7.1	7.7
		Ephemeridae	*Ephemera*	*guttulata*	23.9		28.2
		Heptageniidae	*Cinygmula*	*subaequalis*	26.1	35.7	24.4
			Epeorus	*pleuralis*	28.3	42.9	25.6
			Maccaffertium	*ithaca*	5.4		6.4
				pudicum	21.7	21.4	21.8
		Isonychiidae	*Isonychia*	sp.	43.5	42.9	43.6
Insecta	Hemiptera	Alydidae	*Nariscus*	*fumosus*	6.5	28.6	2.6
		Miridae	*Neolygus*	*omnivagus*	8.7	14.3	7.7
	Hymenoptera	Tenthredinidae	*Craterocercus*	*obtusus*	10.9	14.3	10.3
	Lepidoptera	Erebidae	*Hypena*	*baltimoralis*	12.0	21.4	10.3
			Pharga	*pholausalis*	5.4	28.6	1.3
		Gelechiidae	*Chionodes*	*pereyra*	17.4	28.6	15.4
		Geometridae	*Ectropis*	*crepuscularia*	16.3	21.4	15.4
			Eupithecia	*columbiata*	5.4		6.4
			Lomographa	sp.	10.9	21.4	9.0
			Speranza	*pustularia*	5.4		6.4
		Noctuidae	*Anathix*	*ralla*	12.0	21.4	10.3
			Eupsilia	sp.	25.0	21.4	25.6
			Lithophane	sp.	6.5		7.7
			Orthodes	*cynica*	6.5	14.3	5.1
			Orthosia	*rubescens*	76.1	85.7	74.4

TABLE 3.1. *Continued.*

Class	Order	Family	Genus	Species	% Frequency of Occurrence Overall (n = 92)	LOWA Adults (n = 14)	LOWA Nestlings (n = 78)
Insecta	Lepidoptera	Nymphalidae	*Calisto*	*aquilum*	7.6		9.0
		Tortricidae	*Dichrorampha*	*petiverella*	6.5	7.1	6.4
			Pseudexentera	sp.	28.3	21.4	29.5
				spoliana	9.8	7.1	10.3
	Mecoptera	Bittacidae	*Bittacus*	*pilicornis*	8.7	14.3	7.7
	Megaloptera	Corydalidae	*Nigronia*	*fasciatus*	41.3	50.0	39.7
				serricornis	27.2	28.6	26.9
	Orthoptera	Rhaphidophoridae	*Euhadenoecus*	*puteanus*	23.9	28.6	23.1
	Plecoptera	Capniidae	*Arsapnia*	*coyote*	12.0	28.6	9.0
		Chloroperlidae	*Alloperla*	sp.	12.0	14.3	11.5
				usa	14.1	7.1	15.4
			Haploperla	*brevis*	12.0	28.6	9.0
			Sweltsa	sp.	13.0	21.4	11.5
		Leuctridae	*Leuctra*	sp.	46.7	64.3	43.6
		Nemouridae	*Amphinemura*	sp.	5.4	7.1	5.1
		Perlidae	*Acroneuria*	*carolinensis*	60.9	57.1	61.5
		Perlodidae	*Clioperla*	*clio*	25.0	35.7	23.1
			Isoperla	sp.	37.0	64.3	32.1
		Pteronarcyidae	*Pteronarcys*	*proteus*	32.6	28.6	33.3
	Psocodea	Caeciliusidae	*Valenzuela*	*flavidus*	5.4	14.3	3.8
		Peripsocidae	*Peripsocus*	*subfasciatus*	6.5	21.4	3.8
	Trichoptera	Goeridae	*Goera*	*stylata*	23.9	42.9	20.5
		Limnephilidae	*Limnephilus*	*stigma*	18.5	35.7	15.4
			Pycnopsyche	*gentilis*	13.0	7.1	14.1
				sp.	5.4	7.1	5.1
		Phryganeidae	*Ptilostomis*	*ocellifera*	8.7		10.3
		Rhyacophilidae	*Rhyacophila*	*minora*	7.6	21.4	5.1
				nigrita	9.8	7.1	10.3
Malacostraca	Decapoda	Cambaridae	*Cambarus*	sp.	48.9	57.1	47.4

Overall, 16 orders and 50 families of arthropods were detected across nestling and adult waterthrush diets (Table 3.1). Lepidoptera (100%) and Diptera (97%) were among the most frequently detected arthropod orders in both nestling and adult diets (Table 3.2). Similarly, the pollution-sensitive aquatic orders Ephemeroptera (99%), Plecoptera (91%), and Trichoptera (63%) were among the most frequently detected taxa in both nestling and adult diets (Table 3.2). Terrestrial dietary MOTU richness (total number of identified taxa without an aquatic life-stage) increased significantly as stream pH ($X^2_{5,6} = 8.60$; $P = 0.003$) and EPT availability ($X^2_{5,6} = 5.83$; $P = 0.016$) declined.

While terrestrial arthropods in the orders Orthoptera (Rhaphidophoridae; 24%) and Araneae (Lycosidae; 11%) were uncommon in the diets of Louisiana Waterthrush overall (Table 3.2), logistic regression revealed that the probability of detecting these taxa increased

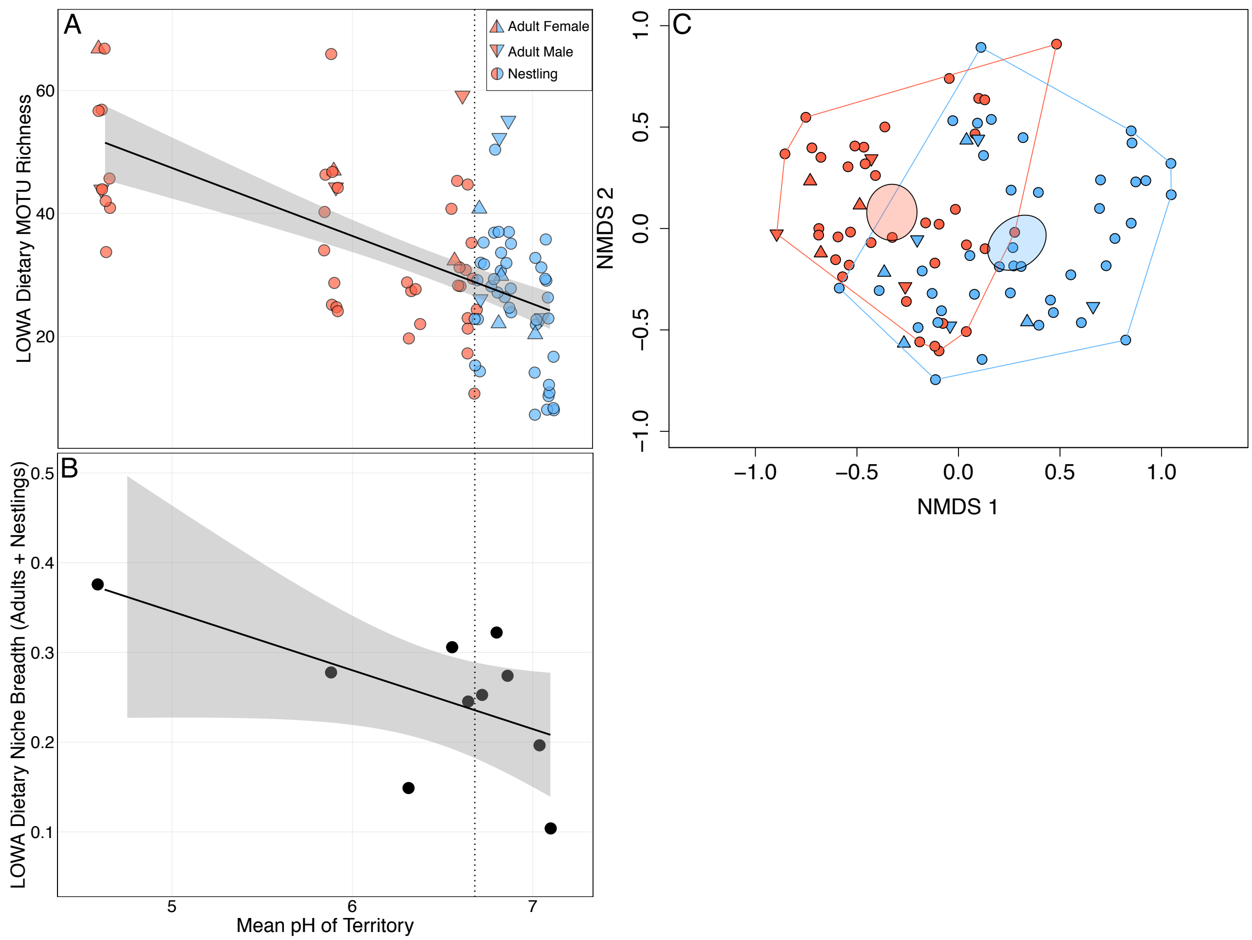

FIGURE 3.1. Shifts in adult and nestling Louisiana Waterthrush (LOWA) diet in response to stream pH. (A) MOTU richness of adult (females = triangles, males = inverted triangles) and nestling (circles) diets increased significantly as mean territory pH declined ($X^2_{5,6}$ = 10.80; *P* = 0.001). Point shading indicates whether a fecal sample was collected from a territory with a pH ≤ (red shading) or > (blue shading) the median value of 6.68 (vertical dotted line). Gray shading around the solid line represents the 95% confidence interval. (B) Total dietary niche breadth (all adults and nestlings associated with a nest) increased significantly ($X^2_{4,5}$ = 4.05; *P* = 0.04) as mean territory pH declined (vertical dotted line = median territory pH of 6.68). (C) Unconstrained NMDS ordination (stress = 0.255) of adult (females = triangles, males = inverted triangles) and nestling (circles) diet composition at the MOTU level. Points represent the taxonomic composition of waterthrush diets and shading indicates that the individual occupied a territory with a pH ≤ (red shading) or > (blue shading) the median value of 6.68. Ellipses represent 95% confidence intervals for group centroids and minimum convex polygons indicate the extent of dietary niche space for each group.

significantly as the availability of EPT taxa declined (Table 3.3). Moreover, the probability of detecting several terrestrial families in the orders Lepidoptera (e.g., Noctuidae; *P* = 0.013), Diptera (e.g., Dolichopodidae; *P* = 0.003), and Mecoptera (Bittacidae; *P* = 0.004) in the diets of waterthrush increased significantly in response to reduced availability of EPT taxa (Table 3.3). For aquatic arthropods, the probability of detecting pollution-tolerant families in the orders

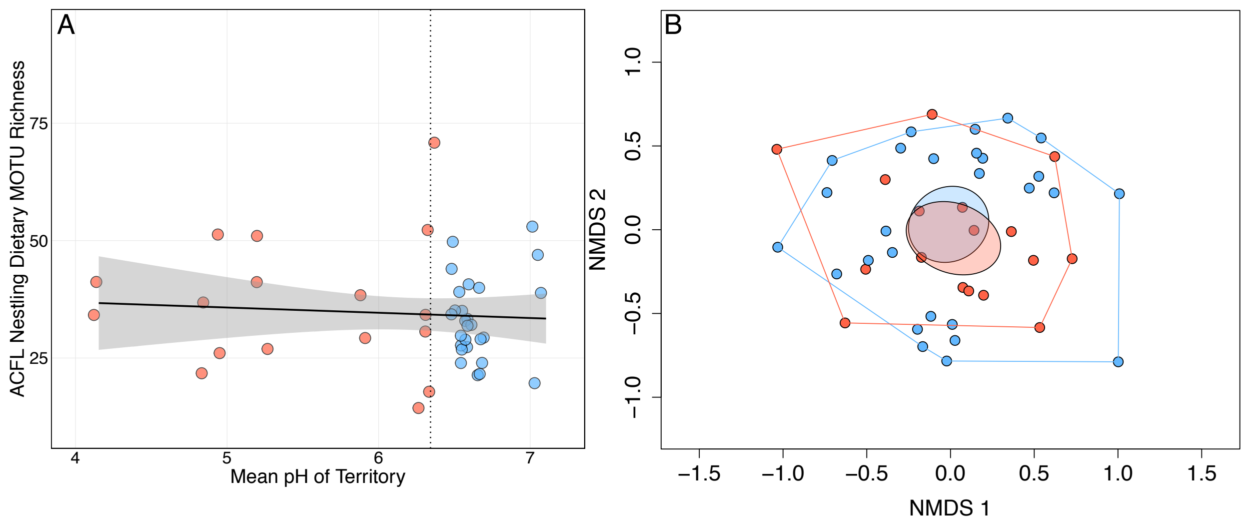

FIGURE 3.2. The diets of Acadian Flycatcher (ACFL) nestlings in riparian habitats are unaffected by reduced stream pH. (A) Dietary MOTU richness of Acadian Flycatcher nestlings did not differ significantly as mean territory pH declined ($X^2_{4,5} = 0.16$; $P = 0.69$). Point shading indicates whether a fecal sample was collected from a territory with a pH ≤ (red shading) or > (blue shading) the median value of 6.34 (vertical dotted line). Gray shading around the solid line represents the 95% confidence interval. (B) Unconstrained NMDS ordination (stress = 0.247) of Acadian Flycatcher nestling diet composition at the MOTU level. Points represent the taxonomic composition of individual diets and shading indicates that the individual occupied a territory with a pH ≤ (red shading) or > (blue shading) the median value of 6.34. Ellipses represent 95% confidence intervals for group centroids and minimum convex polygons indicate the extent of dietary niche space for each group.

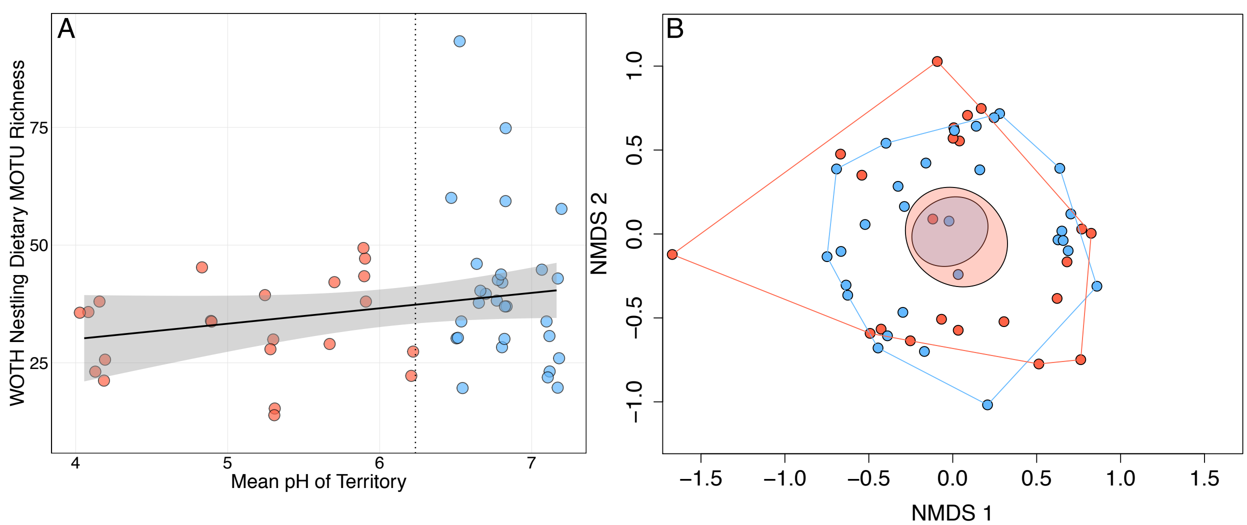

FIGURE 3.3. The diets of Wood Thrush (WOTH) nestlings in riparian habitats are unaffected by reduced stream pH. (A) Dietary MOTU richness of Wood Thrush nestlings did not differ significantly as mean territory pH declined ($X^2_{4,5} = 1.14$; $P = 0.29$). Point shading indicates whether a fecal sample was collected from a territory with a pH ≤ (red shading) or > (blue shading) the median value of 6.24 (vertical dotted line). Gray shading around the solid line represents the 95% confidence interval. (B) Unconstrained NMDS ordination (stress = 0.258) of Wood Thrush nestling diet composition at the MOTU level. Points represent the taxonomic composition of individual diets and shading indicates that the individual occupied a territory with a pH ≤ (red shading) or > (blue shading) the median value of 6.24. Ellipses represent 95% confidence intervals for group centroids and minimum convex polygons indicate the extent of dietary niche space for each group.

Diptera (e.g., Culicidae; $P = 0.009$) and Decapoda (Cambaridae; $P = 0.010$) increased significantly as the availability of EPT taxa declined (Table 3.3). In general, the probability of detecting EPT taxa in waterthrush diets decreased as their availability was reduced (e.g., most Ephemeroptera; Table 3.3); however, the probability of detecting several pollution-sensitive EPT taxa increased significantly (e.g., Ameletidae; $P < 0.001$; Table 3.3) despite their absence from benthic and emergent insect samples collected in acidified territories (Supplemental Tables C.6 and C.7, Appendix C).

3.4 DISCUSSION

In this study, we demonstrated that both adult and nestling Louisiana Waterthrush occupying territories with reduced pH and availability of EPT taxa exhibited significant shifts in diet compared to conspecifics in higher quality territories. These shifts were primarily driven by an expansion of dietary niche breadth (Figures 3.1 and 3.4) resulting from the consumption of terrestrial arthropods such as camel crickets (Orthoptera: Rhaphidophoridae), hangingflies (Mecoptera: Bittacidae), owlet moths (Lepidoptera: Noctuidae), and wolf spiders (Araneae: Lycosidae; Table 3.3), thus providing support for our hypothesis that Louisiana Waterthrush compensate for reduced aquatic prey availability by targeting terrestrial arthropods. Dietary shifts were not observed for other species of Neotropical migrants (Figures 3.2 and 3.3) nesting alongside waterthrush in the same riparian habitat, but primarily consume primarily terrestrial taxa (see Chapter 2). These results suggest that the specialized aquatic foraging strategy utilized by Louisiana Waterthrush renders this species vulnerable to disturbances that compromise stream quality and the availability of pollution-sensitive aquatic taxa.

TABLE 3.2. Percent frequency of occurrence of identified arthropod prey (summarized by order) in the diets of adult and nestling Louisiana Waterthrush. Percent frequency of occurrence = number of fecal samples in which an order was detected divided by the total number of adult and/or nestling fecal samples.

		% Frequency of Occurrence		
Class	**Order**	**Overall (n = 92)**	**LOWA Adults (n = 14)**	**LOWA Nestlings (n = 78)**
Insecta	Lepidoptera	100	100	100
Insecta	Ephemeroptera	99	100	99
Insecta	Diptera	97	100	96
Insecta	Plecoptera	91	100	90
Insecta	Megaloptera	64	79	62
Insecta	Trichoptera	63	79	60
Malacostraca	Decapoda	49	57	47
Insecta	Orthoptera	24	29	23
Insecta	Hemiptera	15	43	10
Insecta	Psocodea	12	36	8
Arachnida	Araneae	11	14	10
Insecta	Hymenoptera	11	14	10
Insecta	Blattodea	9	0	10
Insecta	Mecoptera	9	14	8
Insecta	Coleoptera	8	0	9
Arachnida	Trombidiformes	7	7	6

While Neotropical migrants are known to shift their diets in response to natural fluctuations in prey availability (e.g., Morse 1978; Rodenhouse & Holmes 1992; Rosenberg *et al.* 1982; Rotenberry 1980) and experimental reductions of preferred prey taxa (e.g., Cooper *et al.* 1990; Rodenhouse & Holmes 1992; Sample *et al.* 1993; Whitmore *et al.* 1993), our study is the first to demonstrate that this phenomenon can occur as a result of anthropogenic activities that reduce stream pH or otherwise alter aquatic insect community composition. The observed increase in dietary MOTU richness and total dietary niche breadth suggests that waterthrush compensate for the loss of preferred EPT taxa (see Chapters 1 and 2; Trevelline *et al.* 2016) by

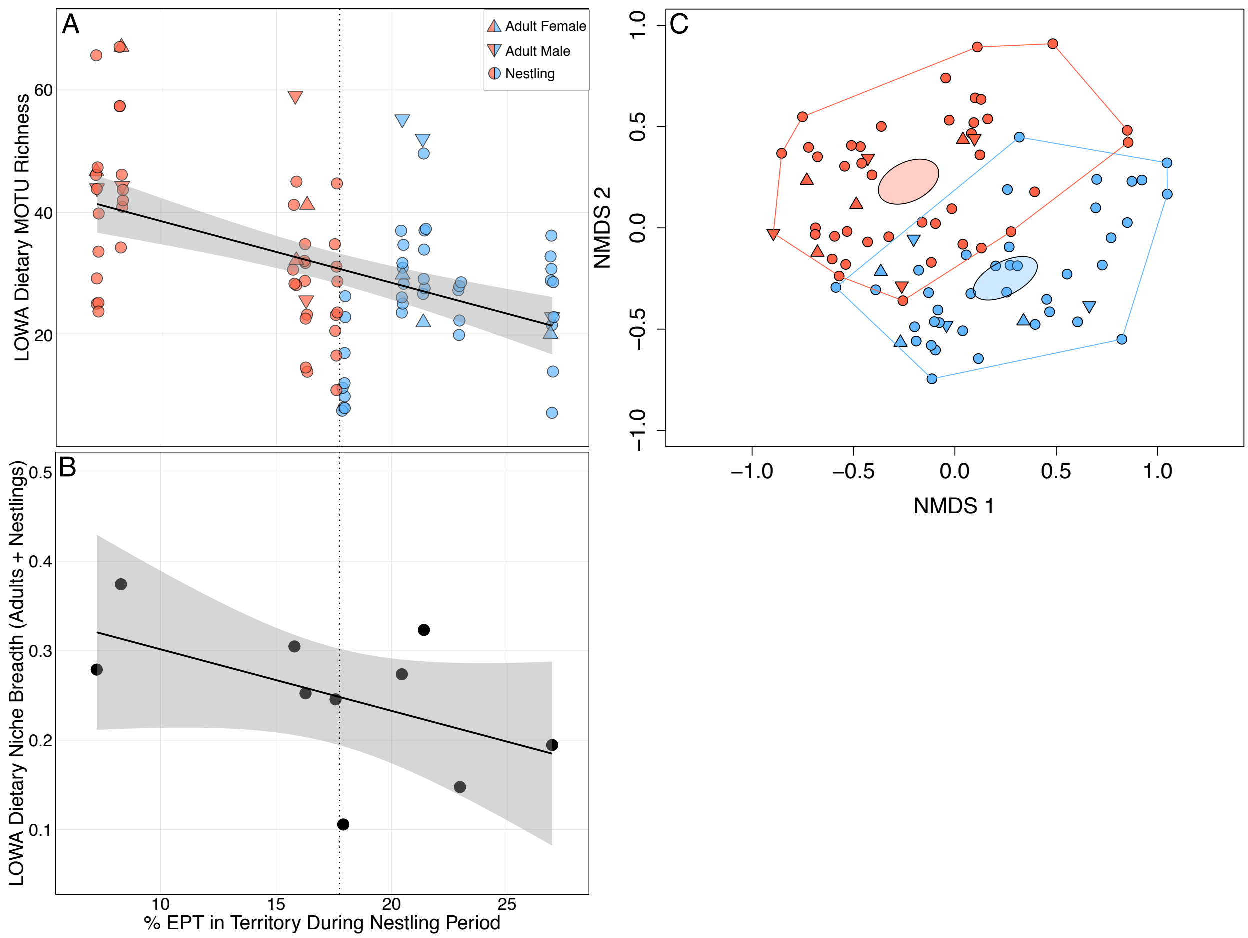

FIGURE 3.4. Shifts in adult and nestling Louisiana Waterthrush (LOWA) diet in response to the availability of EPT taxa during the period of nestling care. (A) MOTU richness of adult (females = triangles, males = inverted triangles) and nestling (circles) diets increased significantly as percent EPT declined ($X^2_{4,5} = 4.97$; $P = 0.026$). Point shading indicates whether a fecal sample was collected from a territory with a percent EPT ≤ (red shading) or > (blue shading) the median value of 17.7 (vertical dotted line). Gray shading around the solid line represents the 95% confidence interval. (B) Total dietary niche breadth (all adults and nestlings associated with a nest) exhibited a marginally significant increase ($X^2_{4,5} = 3.62$; $P = 0.057$) in response to reduced percent EPT (vertical dotted line = median territory percent EPT of 17.7). (C) Unconstrained NMDS ordination (stress = 0.260) of adult (females = triangles, males = inverted triangles) and nestling (circles) diet composition at the MOTU level. Points represent the taxonomic composition of individual diets and shading indicates that the individual occupied a territory with a percent EPT ≤ (red shading) or > (blue shading) the median value of 17.7. Ellipses represent 95% confidence intervals for group centroids and minimum convex polygons indicate the extent of dietary niche space for each group.

altering their foraging behavior. This explanation is consistent with previous studies demonstrating that adult Louisiana Waterthrush breeding in habitats with reduced pH and availability of EPT taxa maintain larger territories and expand their foraging areas to include unimpacted peripheral streams (Mulvihill *et al.* 2008). The expansion of foraging territories in response to habitat degradation has been observed in other species of Neotropical migrants

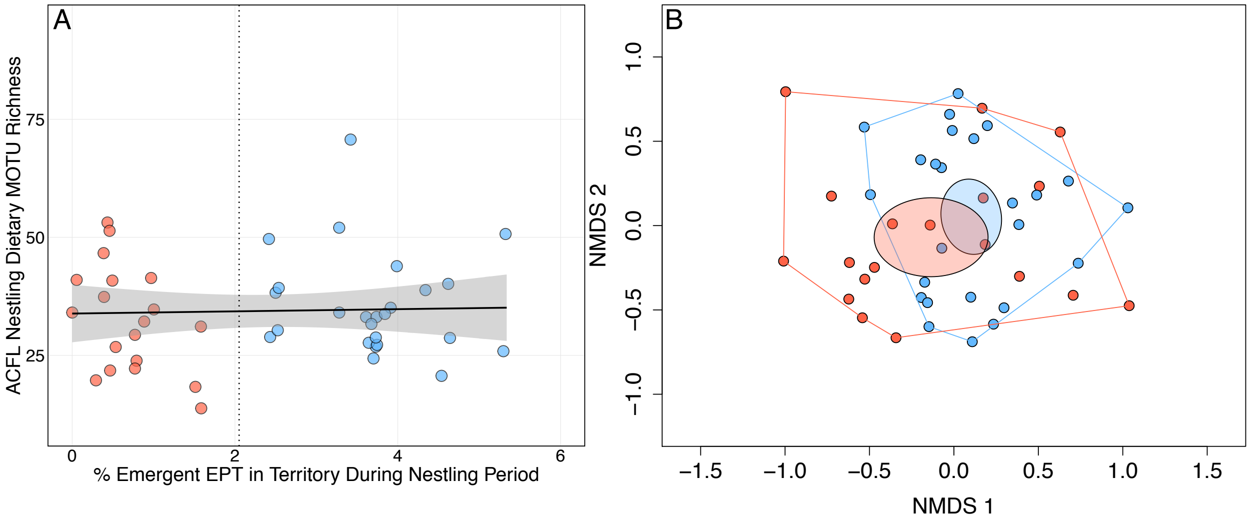

FIGURE 3.5. The diets of Acadian Flycatcher (ACFL) nestlings in riparian habitats are unaffected by reduced EPT availability during the period of nestling care (A) Dietary MOTU richness of Acadian Flycatcher nestlings did not differ significantly as percent EPT declined ($X^2_{4,5} = 0.12$; $P = 0.73$). Point shading indicates whether a fecal sample was collected from a territory with a percent EPT ≤ (red shading) or > (blue shading) the median value of 2.05. (vertical dotted line). Gray shading represents the 95% confidence interval. Gray shading around the solid line represents the 95% confidence interval. (B) Unconstrained NMDS ordination (stress = 0.247) of Acadian Flycatcher nestling diet composition at the MOTU level. Points represent the taxonomic composition of individual diets and shading indicates that the individual occupied a territory with a percent EPT ≤ (red shading) or > (blue shading) the median value of 2.05. Ellipses represent 95% confidence intervals for group centroids and minimum convex polygons indicate the extent of dietary niche space for each group.

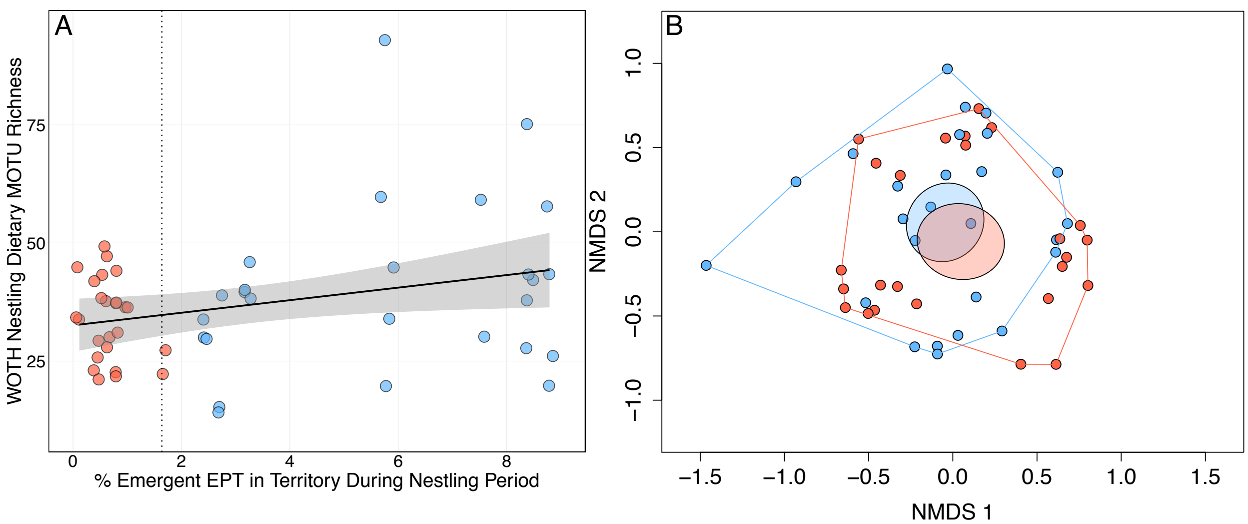

FIGURE 3.6. The diets of Wood Thrush (WOTH) nestlings in riparian habitats are unaffected by reduced EPT availability during the period of nestling care (A) Dietary MOTU richness of Wood Thrush nestlings did not differ significantly as percent EPT declined ($X^2_{4,5} = 2.98$; $P = 0.084$). Point shading indicates whether a fecal sample was collected from a territory with a percent EPT ≤ (red shading) or > (blue shading) the median value of 1.64 (vertical dotted line). Gray shading around the solid line represents the 95% confidence interval. (B) Unconstrained NMDS ordination (stress = 0.260) of Wood Thrush nestling diet composition at the MOTU level. Points represent the taxonomic composition of individual diets and shading indicates that the individual occupied a territory with a percent EPT ≤ (red shading) or > (blue shading) the median value of 1.64. Ellipses represent 95% confidence intervals for group centroids and minimum convex polygons indicate the extent of dietary niche space for each group.

(e.g., Hunter & Witham 1985; Moulding 1976) and in stream-dependent dippers (genus *Cinclus*; Feck & Hall 2004; O'Halloran *et al.* 1990). For waterthrush, such an expansion may provide access to alternative sources of EPT taxa that allow individuals occupying territories along acidified streams to tolerate prey limitations. This explanation is supported by the consumption of several acid-sensitive EPT families (e.g., Ameletidae; Table 3.3) by waterthrush nesting in acidified territories where such taxa were absent from emergent and benthic insect samples (Supplemental Tables C.6 and C.7, Appendix C).

While our results provide evidence that Louisiana Waterthrush are capable of compensating for reduced prey availability by expanding their dietary niche (Figures 3.1 and 3.4) and targeting terrestrial arthropods (Table 3.3), such dietary shifts have the potential to negatively impact reproductive output. For example, experimentally reduced availability of Lepidoptera larvae (preferred prey of most Neotropical migrants during nest provisioning; Holmes *et al.* 1979) resulted in a 3-5 day delay in clutch initiation for breeding Red-eyed Vireos (*Vireo olivaceus*), thus reducing the annual breeding productivity of females (Marshall *et al.* 2002). Similarly, Rodenhouse and Holmes (1992) demonstrated that Black-throated Blue Warblers (*Setophaga caerulescens*) breeding in plots with reduced Lepidoptera availability attempted fewer nests (resulting in fewer fledglings per year). Furthermore, changes in foraging behavior due to reduced prey availability are associated with negative impacts to nestling physiology (Whitmore *et al.* 1993) and survival (Nagy & Smith 1997). Because the expansion of territories has been shown to increase foraging effort and reduce parental care (O'Halloran *et al.* 1990), stream-dependent songbirds may be at greater risk for predation (Martin *et al.* 2000) and brood parasitization (Arcese & Smith 1988), thus reducing nestling survival in acidified habitats and possibly contributing to current population declines (Martin 1987).

TABLE 3.3. Results of logistic regression (using a generalized linear mixed-effects model) for identified arthropod MOTUs (summarized by family) in the diets of adult and nestling Louisiana Waterthrush in response to EPT availability. Only families with significant ($P \leq 0.05$) increases or decreases in probability are reported.

Class	Order	Family	*B*	SD	*P*
Arachnida	Araneae	Lycosidae	-25.98	12.40	0.036
Insecta	Diptera	Culicidae	-34.26	13.04	0.009
		Dolichopodidae	-27.80	9.43	0.003
		Limoniidae	-45.00	16.41	0.006
		Stratiomyidae	-0.73	0.35	0.037
		Syrphidae	-0.56	0.23	0.014
		Tabanidae	-0.67	0.24	0.005
		Tipulidae	-3.63	1.50	0.016
	Ephemeroptera	Ameletidae	-1.70	0.36	< 0.001
		Ephemerellidae	0.69	0.25	0.006
		Ephemeridae	0.70	0.35	0.048
		Isonychiidae	0.71	0.27	0.008
	Lepidoptera	Geometridae	-0.73	0.24	0.002
		Noctuidae	-8.50	3.44	0.013
		Nymphalidae	-1.14	0.39	0.003
	Mecoptera	Bittacidae	-1.00	0.35	0.004
	Megaloptera	Corydalidae	-2.25	0.67	0.001
	Orthoptera	Rhaphidophoridae	-1.43	0.31	< 0.001
	Plecoptera	Capniidae	-0.72	0.29	0.015
		Chloroperlidae	0.78	0.28	0.006
		Leuctridae	-0.75	0.25	0.003
		Perlodidae	-1.08	0.34	0.001
	Trichoptera	Limnephilidae	-1.01	0.26	< 0.001
Malacostraca	Decapoda	Cambaridae	-0.63	0.24	0.010

This study was based on the diets of Louisiana Waterthrush along three streams over the course of a single breeding season. Because diets can vary drastically between locations (e.g., Rotenberry 1980) and years (see differences between waterthrush nestling diets in Chapters 1 and 2), the taxonomic composition of diets presented here should not be considered a fully representative description. Furthermore, the use of a single arthropod-specific PCR primer set prevents the detection of vertebrate taxa thought to be provisioned more frequently by waterthrush nesting along acidified streams (e.g., small fish and salamanders; Mattsson *et al.* 2009; Mulvihill *et al.* 2008). Despite the exclusion of vertebrate prey that would likely increase the magnitude of the dietary shifts, our approach successfully detected significant differences in waterthrush diets as stream pH and EPT availability declined. It is important to note, however, that DNA metabarcoding cannot differentiate between arthropod life-stages (adult and larval insects have identical COI barcode sequences). Therefore, it is impossible to determine (from our data) if waterthrush occupying low-quality habitats further compensate by targeting emergent aquatic insects rather than aquatic larvae, which most likely differ in nutritional content (e.g., Arrese & Soulages 2010) and required handling effort (e.g., Sherry & McDade 1982). Nevertheless, the limitations associated with our approach were consistent across waterthrush fecal samples and should not diminish the conclusions of this study.

In this study, we provide evidence that stream acidification alters the dietary niche of a Neotropical migratory songbird via disruption of aquatic prey resource subsidies. This phenomenon appears to be mediated through the reduced availability of pollution-sensitive EPT taxa, which are vulnerable to a wide-range of anthropogenic activities that affect the chemical or geomorphic profile of aquatic habitats (e.g., Roy *et al.* 2003; Wood *et al.* 2016). Given the increasing frequency and intensity of anthropogenic disturbances in riparian ecosystems (Drohan *et al.* 2012; Dudgeon *et al.* 2006) and the known impact of food limitations on the breeding productivity of Neotropical migrants (reviewed in Martin *et al.* 2000), these activities may negatively impact the conservation of Louisiana Waterthrush or other Neotropical migrants known to opportunistically utilize aquatic insects while breeding in riparian habitats.

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CHAPTER FOUR

Major Conclusions and Future Directions

4.1 CHAPTER ONE: Molecular analysis of nestling diet in a long-distance Neotropical migrant, the Louisiana Waterthrush (*Parkesia motacilla*)

In Chapter One, we successfully developed and applied a next-generation sequencing approach to identify Louisiana Waterthrush prey taxa to the genus or species level. This chapter represents the first published use of DNA metabarcoding to describe the diet of a Neotropical migratory songbird (Trevelline *et al.* 2016). Furthermore, we showed that Louisiana Waterthrush nestling diet was remarkably similar between breeding sites separated by approximately 1,300 km. Importantly, this study was the first to demonstrate the Louisiana Waterthrush nestlings frequently consumed terrestrial Lepidoptera, which are easily digested and may have been overlooked in previous diet studies that relied on morphological identification (e.g., Eaton 1958). This finding changed our view of how this riparian-obligate songbird utilizes food resources during the period of nestling care, thus highlighting the benefits of studying passerine diets using DNA metabarcoding.

While several potential future directions of this work have already been explored in subsequent studies (see Chapters 2 and 3), the diets of adult waterthrush in non-breeding habitats remain poorly understood, and thus is a promising direction for future diet studies. In general, arthropod availability on the Neotropical wintering grounds (e.g., the Caribbean) is substantially more limited than during the breeding season (Sherry *et al.* 2005). Such food limitations are especially prominent during annual droughts that typically occur just before spring migration (February-March), which is a critical period when migrants must deposit enough fat to successfully traverse the Gulf of Mexico (Katti & Price 1999; Moore & Kerlinger 1987). Little is known regarding the diets of Neotropical migrants during this period and DNA metabarcoding could provide a detailed description of waterthrush diets during a period critical to maintaining population stability (Sherry *et al.* 2005). Understanding waterthrush diet on the wintering grounds may be particularly valuable given that predicted drying trends in the Caribbean (Karmalkar *et al.* 2013) are likely to exacerbate current arthropods declines that are already affecting several species of Neotropical migrants (e.g., Strong & Sherry 2001; Studds & Marra 2007, 2011). Furthermore, contrasting the diet of waterthrush in breeding and non-breeding conditions would provide a better understanding of how these birds (and Neotropical migrants in general) modify their diets under changing environmental conditions over the course of the annual cycle.

4.2 CHAPTER TWO: DNA metabarcoding of nestling feces reveals provisioning of aquatic prey and resource partitioning among Neotropical migratory songbirds in a riparian habitat

In Chapter Two, we applied our DNA metabarcoding technique (developed in Chapter 1; Trevelline *et al.* 2016) to investigate a topic that has intrigued ecologists for nearly 60 years (MacArthur 1958): resource partitioning among breeding Neotropical migratory birds. Using our molecular approach and multivariate statistical analyses, we revealed significant interspecific dietary niche divergence among three syntopic species—Acadian Flycatcher, Louisiana Waterthrush, and Wood Thrush—sharing a common riparian habitat during breeding. Furthermore, we found that these species frequently consumed aquatic arthropods, emphasizing the importance of aquatic resource subsidies to terrestrial predators with substantial differences in foraging strategies.

The use of DNA metabarcoding to investigate dietary niche partitioning among syntopic warblers during the non-breeding season represents an exciting direction for future studies. Because riparian zones are often characterized by an abundance of arthropod prey (e.g., Nakano

& Murakami 2001), interspecific competition arising from food limitations is not a likely outcome (reviewed in Martin 1987). On the wintering grounds of the Caribbean, however, migratory birds must co-exist at much higher densities than on the breeding grounds, leading to competition for prey resources during the most food-limited period of the annual cycle (Newton 2004). The study of diets in such a study system using DNA metabarcoding has the potential to reveal interspecific competition for limited prey resources among species with foraging strategies that are difficult to observe (e.g., Swainson's Warbler; Strong 2000).

4.3 CHAPTER THREE: Stream acidification and reduced availability of pollution-sensitive aquatic prey alter the dietary niche of a stream-dependent Neotropical migratory songbird

In Chapter Three, we utilized DNA metabarcoding to provide evidence that both adult and nestling Louisiana Waterthrush occupying territories with reduced pH and availability of EPT taxa compensate by targeting terrestrial prey, resulting in significant shifts in diet compared to conspecifics in higher quality territories. In addition to providing support for our hypothesis that Louisiana Waterthrush compensate for food shortages by targeting terrestrial arthropods in degraded riparian habitats, our findings emphasize the vulnerability of Louisiana Waterthrush to anthropogenic disturbances that compromise stream quality and the availability of pollution-sensitive aquatic insects. While Robert Mulvihill first proposed this hypothesis based on field observations at Powdermill Nature Reserve nearly 20 years ago, the methods used to conduct this study (Illumina sequencing and computational bioinformatics) have only been available for the last several years. Furthermore, the complexity of sequencing heavily degraded residual prey DNA from the feces of passerines presented serious challenges best evidenced by the dearth of such studies in academic literature (only 3 studies to date; Crisol-Martínez *et al.* 2016; Jedlicka *et al.* 2016; Trevelline *et al.* 2016). The novelty of these approaches combined with the logistical (e.g., 3 month field seasons) and technical challenges (e.g., DNA extraction from uric-acid rich feces, DNA degradation) presented by this study required the help of numerous field technicians (see acknowledgements) and nearly 6 years of laboratory-based molecular investigation.

Future studies should investigate the potential impact of shifts in diet on nestling physiology and survival. For example, investigating how changes in diet alter the gut microbiome appears to be a particularly promising avenue of research. Recent evidence suggests that the gut microbiome is highly responsive to changes in diet (Costello *et al.* 2012), and thus would be expected to differ substantially between waterthrush occupying acidified versus circumneutral territories. Because the gut microbiome is essential to several important physiological processes (e.g., development and lipid metabolism; Ley *et al.* 2006; Sommer & Bäckhed 2013), changes in gut microbial communities may represent an undescribed mechanism affecting waterthrush survival and the long-term conservation of Neotropical migrants in general.

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APPENDIX A

Protocol for the extraction of prey DNA from avian feces

This protocol is designed to maximize extraction of insect prey DNA from bird feces stored in ethanol. It does not prevent or exclude the extraction of bird, bacterial, fungal, or other non-prey DNA from fecal samples. DNA extraction from avian fecal material using Qiagen QIAamp DNA Stool Kit (Cat. #: 51504). Adapted from Zeale et al. (2011) and Qiagen Handbook August 2001: Protocol for Isolation of DNA from stool for Pathogen Detection.

DAY 1:

1. Transfer fecal sample (including preservative ethanol) into a sterile weigh boat.

2. Homogenize fecal sample using a sterile, DNA-free instrument (e.g. pipette tip) to permit complete ethanol evaporation.

3. Incubate fecal sample in weigh boat using a slide warmer set to medium heat. Incubate until sample is completely dry and all ethanol has evaporated (~1 hour). Residual ethanol will interfere with DNA extraction.

4. Carefully transfer as much of the dried fecal material as possible to a sterile 2 mL microcentrifuge tube. Add 1.4 mL ASL buffer to the weigh boat to transfer any remaining fecal material. Continuously vortex the sample for 10 minutes.

5. Incubate the suspension overnight at 70°C, vortexing occasionally.

DAY 2:

6. Vortex continuously for 10 minutes and centrifuge at full speed (~20,000 x g) for 1 minute at room temperature to pellet fecal particulate.

7. Pipet 1.2 ml of the supernatant into a new 2 ml centrifuge tube.

8. Add 1 InhibitEX tablet to the sample and vortex immediately and continuously for 3 minutes or until completely suspended. Incubate suspension for 5 minutes at room temperature to allow inhibitors to absorb to the InhibitEX matrix.

9. Centrifuge sample at full speed for 3 minutes to pellet InhibitEX matrix.

10. Transfer 600 µl of supernatant into a new 1.5 ml centrifuge tube and discard the pellet.

11. Add 40 µL Proteinase K the supernatant and mix thoroughly by vortexing.

12. Add 600 µl Buffer AL and vortex for 15 seconds. Incubate overnight at 70°C.

DAY 3

13. Remove sample from incubation and vortex continuously for 1 minute.

14. Add 600 µl of 100% ethanol to the lysate and mix by vortexing.

15. Add 600µl of the lysate to a QIAmp spin column. Centrifuge at full speed for 1 minute. Place spin column in a new collection tube and discard the tube containing the filtrate.

16. Repeat step 13 to load the remaining aliquots of the lysate to the spin column.

17. Add 500 µl Buffer AW1. Centrifuge at full speed for 1 minute. Place spin column in a new collection tube and discard the tube containing the filtrate.

18. Add 500 µl Buffer AW2. Centrifuge at full speed for 3 minutes. Place spin column in into a new 1.5 ml centrifuge tube and discard the tube containing the filtrate

19. Pipette 50µl of pre-warmed (70°C) Buffer AE directly onto the spin column membrane. Incubate for 5 minutes at room temperature then centrifuge at full speed for 1 minute to elute DNA.

20. Transfer the eluted DNA from step 19 onto the spin column membrane to concentrate the DNA sample. Incubate for 2 minutes and centrifuge at full speed for 1 minute.

APPENDIX B

COI barcode identification protocol using the BOLD reference database

MOTU identification criteria using the BOLD search tool (species-level barcode records). Adapted from Razgour et al. (2011).

1. 100% match to one species – species-level assignment, or 100% match to more than one species that belong to the same genus: genus-level assignment.

2. > 98% match to one species – species-level assignment, or > 98% match to more than one species that belong to the same genus: genus-level assignment

3. > 98% match to one or more taxa (genus or species) in the same family: family-level assignment

4. > 98% match to one or more taxa (genus, species, or family) to in the same order: order-level assignment.

5. < 98% match to one or more taxa: Assignment to most conservative taxonomic level.

6. No match in BOLD.

APPENDIX C

Sample, nest, and territory metadata

SUPPLEMENTAL TABLE C.1. Louisiana Waterthrush fecal sample metadata for Chapter 1.

SAMPLE_ID	NEST_ID	STREAM	COLLECTION_DATE
AR1_1	AR1	Sis Hollow	14-May-2013
AR1_2	AR1	Sis Hollow	14-May-2013
AR1_3	AR1	Sis Hollow	14-May-2013
AR1_4	AR1	Sis Hollow	14-May-2013
AR1_5	AR1	Sis Hollow	14-May-2013
AR2_1	AR2	Sis Hollow	15-May-2013
AR2_2	AR2	Sis Hollow	15-May-2013
AR2_3	AR2	Sis Hollow	15-May-2013
AR2_4	AR2	Sis Hollow	15-May-2013
AR3_1	AR3	E Point Remove Creek	15-May-2013
AR3_2	AR3	E Point Remove Creek	15-May-2013
AR3_3	AR3	E Point Remove Creek	15-May-2013
AR3_4	AR3	E Point Remove Creek	15-May-2013
AR3_5	AR3	E Point Remove Creek	15-May-2013
AR4_1	AR4	Sunnyside Creek	17-May-2013
AR4_2	AR4	Sunnyside Creek	17-May-2013
AR5_1	AR5	Sis Hollow	18-May-2013
AR5_2	AR5	Sis Hollow	18-May-2013
AR5_3	AR5	Sis Hollow	18-May-2013
AR5_4	AR5	Sis Hollow	18-May-2013
AR6_1	AR6	E Point Remove Creek	20-May-2013
AR6_2	AR6	E Point Remove Creek	20-May-2013
AR6_3	AR6	E Point Remove Creek	20-May-2013
AR6_4	AR6	E Point Remove Creek	20-May-2013
AR6_5	AR6	E Point Remove Creek	20-May-2013
AR7_1	AR7	Sunnyside Creek	22-May-2013
AR7_2	AR7	Sunnyside Creek	22-May-2013
AR8_1	AR8	Cedar Creek	26-May-2013
AR8_2	AR8	Cedar Creek	26-May-2013
AR8_3	AR8	Cedar Creek	26-May-2013
AR9_1	AR9	E Point Remove Creek	31-May-2013
AR9_2	AR9	E Point Remove Creek	31-May-2013
AR9_3	AR9	E Point Remove Creek	31-May-2013
AR10_1	AR10	Sunnyside Creek	1-Jun-2013
AR11_1	AR11	E Point Remove Creek	3-Jun-2013
AR11_2	AR11	E Point Remove Creek	3-Jun-2013
AR11_3	AR11	E Point Remove Creek	3-Jun-2013
AR12_1	AR12	Sunnyside Creek	7-Jun-2013
AR13_1	AR13	Cedar Creek	11-Jun-2013
AR13_2	AR13	Cedar Creek	11-Jun-2013
AR13_3	AR13	Cedar Creek	11-Jun-2013

SUPPLEMENTAL TABLE C.1. *Continued.*

SAMPLE_ID	NEST_ID	STREAM	COLLECTION_DATE
AR14_1	AR14	Sunnyside Creek	19-Jun-2013
AR14_2	AR14	Sunnyside Creek	19-Jun-2013
AR14_3	AR14	Sunnyside Creek	19-Jun-2013
AR15_1	AR15	Cedar Creek	29-Jun-2013
AR15_2	AR15	Cedar Creek	29-Jun-2013
AR15_3	AR15	Cedar Creek	29-Jun-2013
AR16_1	AR16	Sunnyside Creek	4-Jun-2013
PA1_1	PA1	Loyalhanna Creek	15-May-2013
PA1_2	PA1	Loyalhanna Creek	15-May-2013
PA1_3	PA1	Loyalhanna Creek	15-May-2013
PA1_4	PA1	Loyalhanna Creek	15-May-2013
PA1_5	PA1	Loyalhanna Creek	15-May-2013
PA1_6	PA1	Loyalhanna Creek	15-May-2013
PA2_1	PA2	Camp Run	20-May-2013
PA2_2	PA2	Camp Run	20-May-2013
PA2_3	PA2	Camp Run	20-May-2013
PA2_4	PA2	Camp Run	20-May-2013
PA2_5	PA2	Camp Run	20-May-2013
PA3_1	PA3	Linn Run	20-May-2013
PA3_2	PA3	Linn Run	20-May-2013
PA3_3	PA3	Linn Run	20-May-2013
PA3_4	PA3	Linn Run	20-May-2013
PA3_5	PA3	Linn Run	20-May-2013
PA4_1	PA4	Linn Run	22-May-2013
PA4_2	PA4	Linn Run	22-May-2013
PA4_3	PA4	Linn Run	22-May-2013
PA4_4	PA4	Linn Run	22-May-2013
PA4_5	PA4	Linn Run	22-May-2013
PA5_1	PA5	Camp Run	23-May-2013
PA5_2	PA5	Camp Run	23-May-2013
PA5_3	PA5	Camp Run	23-May-2013
PA5_4	PA5	Camp Run	23-May-2013
PA5_5	PA5	Camp Run	23-May-2013
PA6_1	PA6	Powdermill Run	24-May-2013
PA6_2	PA6	Powdermill Run	24-May-2013
PA6_3	PA6	Powdermill Run	24-May-2013
PA6_4	PA6	Powdermill Run	24-May-2013
PA6_5	PA6	Powdermill Run	24-May-2013
PA7_2	PA7	Powdermill Run	24-May-2013
PA7_3	PA7	Powdermill Run	24-May-2013
PA7_4	PA7	Powdermill Run	24-May-2013

SUPPLEMENTAL TABLE C.1. *Continued.*

SAMPLE_ID	NEST_ID	STREAM	COLLECTION_DATE
PA8_1	PA8	Powdermill Run	25-May-2013
PA8_2	PA8	Powdermill Run	25-May-2013
PA8_3	PA8	Powdermill Run	25-May-2013
PA8_5	PA8	Powdermill Run	25-May-2013
PA9_1	PA9	Linn Run	27-May-2013
PA9_2	PA9	Linn Run	27-May-2013
PA9_3	PA9	Linn Run	27-May-2013
PA9_4	PA9	Linn Run	27-May-2013
PA9_5	PA9	Linn Run	27-May-2013
PA10_1	PA10	Linn Run	27-May-2013
PA10_2	PA10	Linn Run	27-May-2013
PA10_3	PA10	Linn Run	27-May-2013
PA10_4	PA10	Linn Run	27-May-2013
PA10_5	PA10	Linn Run	27-May-2013
PA11_1	PA11	Linn Run	27-May-2013
PA11_2	PA11	Linn Run	27-May-2013
PA11_3	PA11	Linn Run	27-May-2013
PA11_4	PA11	Linn Run	27-May-2013
PA11_5	PA11	Linn Run	27-May-2013
PA12_1	PA12	Linn Run	29-May-2013
PA12_2	PA12	Linn Run	29-May-2013
PA12_3	PA12	Linn Run	29-May-2013
PA13_1	PA13	Powdermill Run	30-May-2013
PA13_2	PA13	Powdermill Run	30-May-2013
PA13_5	PA13	Powdermill Run	30-May-2013
PA14_1	PA14	Loyalhanna Creek	6-Jun-2013
PA14_2	PA14	Loyalhanna Creek	6-Jun-2013
PA14_3	PA14	Loyalhanna Creek	6-Jun-2013
PA14_4	PA14	Loyalhanna Creek	6-Jun-2013
PA14_5	PA14	Loyalhanna Creek	6-Jun-2013
PA15_1	PA15	Powdermill Run	6-Jun-2013
PA15_2	PA15	Powdermill Run	6-Jun-2013
PA15_3	PA15	Powdermill Run	6-Jun-2013
PA15_4	PA15	Powdermill Run	6-Jun-2013
PA15_5	PA15	Powdermill Run	6-Jun-2013
PA15_6	PA15	Powdermill Run	6-Jun-2013
PA16_1	PA16	Linn Run	24-Jun-2013
PA16_2	PA16	Linn Run	24-Jun-2013
PA16_3	PA16	Linn Run	24-Jun-2013
PA16_4	PA16	Linn Run	24-Jun-2013
PA16_5	PA16	Linn Run	24-Jun-2013

SUPPLEMENTAL TABLE C.2. Riparian nestling fecal sample metadata for Chapter 2.

SAMPLE_ID	NEST_ID	SPECIES	STREAM	DATE_COLLECTED
DAM42.1	DAM42	ACFL	LAUREL	12-Jun-2015
DAM42.2	DAM42	ACFL	LAUREL	12-Jun-2015
BKT21.1	BKT21	ACFL	LAUREL	15-Jun-2015
BKT21.2	BKT21	ACFL	LAUREL	15-Jun-2015
BKT44.1	BKT44	ACFL	LAUREL	15-Jun-2015
BDH29.1	BDH29	ACFL	LAUREL	17-Jun-2015
BDH29.2	BDH29	ACFL	LAUREL	17-Jun-2015
BDH29.3	BDH29	ACFL	LAUREL	17-Jun-2015
DAM32.1	DAM32	ACFL	LAUREL	29-Jun-2015
DAM32.2	DAM32	ACFL	LAUREL	29-Jun-2015
DAM40.1	DAM40	ACFL	LAUREL	24-Jul-2015
DAM40.2	DAM40	ACFL	LAUREL	24-Jul-2015
BKT48.1	BKT48	ACFL	LOYAL	17-Jun-2015
DAM39.1	DAM39	ACFL	LOYAL	20-Jul-2015
DAM39.2	DAM39	ACFL	LOYAL	20-Jul-2015
DAM39.3	DAM39	ACFL	LOYAL	20-Jul-2015
BKT17.1	BKT17	ACFL	POWD	15-Jun-2015
BKT17.2	BKT17	ACFL	POWD	15-Jun-2015
BKT17.3	BKT17	ACFL	POWD	15-Jun-2015
BKT17.4	BKT17	ACFL	POWD	15-Jun-2015
BKT17.5	BKT17	ACFL	POWD	15-Jun-2015
BKT24.1	BKT24	ACFL	POWD	15-Jun-2015
BKT24.2	BKT24	ACFL	POWD	15-Jun-2015
BKT24.3	BKT24	ACFL	POWD	15-Jun-2015
BKT27.1	BKT27	ACFL	POWD	17-Jun-2015
BKT27.2	BKT27	ACFL	POWD	17-Jun-2015
BKT27.3	BKT27	ACFL	POWD	17-Jun-2015
BKT25.1	BKT25	ACFL	POWD	18-Jun-2015
BKT25.2	BKT25	ACFL	POWD	18-Jun-2015
BKT25.3	BKT25	ACFL	POWD	18-Jun-2015
DAM12.1	DAM12	ACFL	POWD	18-Jun-2015
DAM12.2	DAM12	ACFL	POWD	18-Jun-2015
DAM12.3	DAM12	ACFL	POWD	18-Jun-2015
BKT64.1	BKT64	ACFL	POWD	17-Jul-2015
BKT64.2	BKT64	ACFL	POWD	17-Jul-2015
BKT64.3	BKT64	ACFL	POWD	17-Jul-2015
DAM34.1	DAM34	ACFL	POWD	20-Jul-2015
DAM34.2	DAM34	ACFL	POWD	20-Jul-2015
DAM35.1	DAM35	ACFL	POWD	20-Jul-2015
DAM35.2	DAM35	ACFL	POWD	20-Jul-2015
DAM35.3	DAM35	ACFL	POWD	20-Jul-2015

SUPPLEMENTAL TABLE C.2. *Continued.*

SAMPLE_ID	NEST_ID	SPECIES	STREAM	DATE_COLLECTED
BDH21.1	BDH21	AFCL	POWD	17-Jun-2015
BDH21.2	BDH21	AFCL	POWD	17-Jun-2015
BDH21.3	BDH21	AFCL	POWD	17-Jun-2015
BKT3.1	BKT3	LOWA	LAUREL	27-May-2015
BKT3.2	BKT3	LOWA	LAUREL	27-May-2015
BKT3.3	BKT3	LOWA	LAUREL	27-May-2015
BKT3.4	BKT3	LOWA	LAUREL	27-May-2015
BKT3.5	BKT3	LOWA	LAUREL	27-May-2015
BKT1.10	BKT1	LOWA	LOYAL	20-May-2015
BKT1.11	BKT1	LOWA	LOYAL	20-May-2015
BKT1.6	BKT1	LOWA	LOYAL	20-May-2015
BKT1.7	BKT1	LOWA	LOYAL	20-May-2015
BKT1.8	BKT1	LOWA	LOYAL	20-May-2015
BKT2.10	BKT2	LOWA	LOYAL	25-May-2015
BKT2.6	BKT2	LOWA	LOYAL	25-May-2015
BKT2.7	BKT2	LOWA	LOYAL	25-May-2015
BKT2.8	BKT2	LOWA	LOYAL	25-May-2015
BKT2.9	BKT2	LOWA	LOYAL	25-May-2015
DAM1.4	DAM1	LOWA	POWD	22-May-2015
DAM1.5	DAM1	LOWA	POWD	22-May-2015
DAM1.6	DAM1	LOWA	POWD	22-May-2015
MMP1.1	MMP1	LOWA	POWD	25-May-2015
MMP1.2	MMP1	LOWA	POWD	25-May-2015
MMP1.3	MMP1	LOWA	POWD	25-May-2015
MMP1.4	MMP1	LOWA	POWD	25-May-2015
BKT5.5	BKT5	LOWA	POWD	28-May-2015
BKT5.6	BKT5	LOWA	POWD	28-May-2015
BKT5.7	BKT5	LOWA	POWD	28-May-2015
BKT5.8	BKT5	LOWA	POWD	28-May-2015
BKT5.9	BKT5	LOWA	POWD	28-May-2015
BKT7.5	BKT7	LOWA	POWD	28-May-2015
BKT7.6	BKT7	LOWA	POWD	28-May-2015
BKT7.7	BKT7	LOWA	POWD	28-May-2015
BKT7.8	BKT7	LOWA	POWD	28-May-2015
MMP2.6	MMP2	LOWA	POWD	28-May-2015
MMP2.7	MMP2	LOWA	POWD	28-May-2015
MMP2.8	MMP2	LOWA	POWD	28-May-2015
MMP2.9	MMP2	LOWA	POWD	28-May-2015
BKT9.5	BKT9	LOWA	POWD	1-Jun-2015
BKT9.6	BKT9	LOWA	POWD	1-Jun-2015
BKT9.7	BKT9	LOWA	POWD	1-Jun-2015

SUPPLEMENTAL TABLE C.2. *Continued.*

SAMPLE_ID	NEST_ID	SPECIES	STREAM	DATE_COLLECTED
BKT9.8	BKT9	LOWA	POWD	1-Jun-2015
BDH14.1	BDH14	WOTH	LAUREL	2-Jun-2015
BDH14.2	BDH14	WOTH	LAUREL	2-Jun-2015
BDH14.3	BDH14	WOTH	LAUREL	2-Jun-2015
BDH14.4	BDH14	WOTH	LAUREL	2-Jun-2015
BKT43.1	BKT43	WOTH	LAUREL	11-Jun-2015
BKT43.2	BKT43	WOTH	LAUREL	11-Jun-2015
BDH26.1	BDH26	WOTH	LAUREL	17-Jun-2015
BDH26.2	BDH26	WOTH	LAUREL	17-Jun-2015
BDH26.3	BDH26	WOTH	LAUREL	17-Jun-2015
BDH26.4	BDH26	WOTH	LAUREL	22-Jun-2015
MMP7.1	MMP7	WOTH	LAUREL	22-Jun-2015
MMP7.2	MMP7	WOTH	LAUREL	22-Jun-2015
MMP7.3	MMP7	WOTH	LAUREL	22-Jun-2015
BKT56.3	BKT56	WOTH	LAUREL	9-Jul-2015
BKT56.4	BKT56	WOTH	LAUREL	9-Jul-2015
BKT56.5	BKT56	WOTH	LAUREL	9-Jul-2015
DAM61.1	DAM61	WOTH	LAUREL	18-Jul-2015
DAM61.2	DAM61	WOTH	LAUREL	18-Jul-2015
DAM52.1	DAM52	WOTH	LAUREL	24-Jul-2015
DAM52.2	DAM52	WOTH	LAUREL	24-Jul-2015
DAM50.1	DAM50	WOTH	LAUREL	1-Aug-2015
DAM50.2	DAM50	WOTH	LAUREL	1-Aug-2015
BDH22.1	BDH22	WOTH	LOYAL	4-Jun-2015
BDH22.2	BDH22	WOTH	LOYAL	4-Jun-2015
DAM6.1	DAM6	WOTH	LOYAL	4-Jun-2015
DAM6.2	DAM6	WOTH	LOYAL	4-Jun-2015
DAM6.3	DAM6	WOTH	LOYAL	4-Jun-2015
DAM6.4	DAM6	WOTH	LOYAL	4-Jun-2015
BKT47.1	BKT47	WOTH	LOYAL	7-Jul-2015
BKT47.2	BKT47	WOTH	LOYAL	7-Jul-2015
BKT47.3	BKT47	WOTH	LOYAL	7-Jul-2015
BDH5.1	BDH5	WOTH	POWD	29-May-2015
BDH5.2	BDH5	WOTH	POWD	29-May-2015
BDH5.3	BDH5	WOTH	POWD	29-May-2015
BKT4.1	BKT4	WOTH	POWD	29-May-2015
BKT4.2	BKT4	WOTH	POWD	29-May-2015
BKT4.3	BKT4	WOTH	POWD	29-May-2015
BKT4.4	BKT4	WOTH	POWD	29-May-2015
BKT4.5	BKT4	WOTH	POWD	29-May-2015
BKT6.1	BKT6	WOTH	POWD	29-May-2015

SUPPLEMENTAL TABLE C.2. *Continued.*

SAMPLE_ID	NEST_ID	SPECIES	STREAM	DATE_COLLECTED
BKT6.2	BKT6	WOTH	POWD	29-May-2015
BKT6.3	BKT6	WOTH	POWD	29-May-2015
BKT6.4	BKT6	WOTH	POWD	29-May-2015
BDH10.1	BDH10	WOTH	POWD	10-Jun-2015
BDH10.2	BDH10	WOTH	POWD	10-Jun-2015
BKT39.1	BKT39	WOTH	POWD	28-Jun-2015
BKT39.2	BKT39	WOTH	POWD	28-Jun-2015
BKT39.3	BKT39	WOTH	POWD	28-Jun-2015
BKT62.1	BKT62	WOTH	POWD	13-Jul-2015
BKT62.2	BKT62	WOTH	POWD	13-Jul-2015
BKT62.3	BKT62	WOTH	POWD	13-Jul-2015

SUPPLEMENTAL TABLE C.3. Louisiana Waterthrush fecal sample metadata for Chapter 3.

SAMPLE_ID	NEST_ID	STREAM	FLAG	AGE_SEX	COLLECTION_DATE	PERCENT_EPT	AVG_PH	MOTU_RICHNESS
BKT1_1	BKT1	LOYAL	54	Nestling	18-May-2015	0.2695	7.0441	36
BKT1_10	BKT1	LOYAL	54	Nestling	20-May-2015	0.2695	7.0441	33
BKT1_11	BKT1	LOYAL	54	Nestling	20-May-2015	0.2695	7.0441	7
BKT1_12	BKT1	LOYAL	54	AdultMale	20-May-2015	0.2695	7.0441	23
BKT1_13	BKT1	LOYAL	54	AdultFemale	20-May-2015	0.2695	7.0441	20
BKT1_2	BKT1	LOYAL	54	Nestling	18-May-2015	0.2695	7.0441	31
BKT1_4	BKT1	LOYAL	54	Nestling	18-May-2015	0.2695	7.0441	29
BKT1_5	BKT1	LOYAL	54	Nestling	18-May-2015	0.2695	7.0441	22
BKT1_6	BKT1	LOYAL	54	Nestling	20-May-2015	0.2695	7.0441	14
BKT1_7	BKT1	LOYAL	54	Nestling	20-May-2015	0.2695	7.0441	23
BKT1_8	BKT1	LOYAL	54	Nestling	20-May-2015	0.2695	7.0441	29
BKT14_1	BKT14	POWD	28 TRIB	Nestling	3-Jun-2015	0.0829	4.6273	57
BKT14_10	BKT14	POWD	28 TRIB	AdultMale	1-Jun-2015	0.0829	4.6273	44
BKT14_2	BKT14	POWD	28 TRIB	Nestling	3-Jun-2015	0.0829	4.6273	57
BKT14_3	BKT14	POWD	28 TRIB	Nestling	3-Jun-2015	0.0829	4.6273	44
BKT14_4	BKT14	POWD	28 TRIB	Nestling	3-Jun-2015	0.0829	4.6273	34
BKT14_5	BKT14	POWD	28 TRIB	Nestling	1-Jun-2015	0.0829	4.6273	41
BKT14_6	BKT14	POWD	28 TRIB	Nestling	1-Jun-2015	0.0829	4.6273	46
BKT14_7	BKT14	POWD	28 TRIB	Nestling	1-Jun-2015	0.0829	4.6273	42
BKT14_8	BKT14	POWD	28 TRIB	Nestling	1-Jun-2015	0.0829	4.6273	67
BKT14_9	BKT14	POWD	28 TRIB	AdultFemale	1-Jun-2015	0.0829	4.6273	67
BKT2_1	BKT2	LOYAL	12	Nestling	26-May-2015	0.1791	7.0973	23
BKT2_10	BKT2	LOYAL	12	Nestling	25-May-2015	0.1791	7.0973	26
BKT2_2	BKT2	LOYAL	12	Nestling	26-May-2015	0.1791	7.0973	11
BKT2_4	BKT2	LOYAL	12	Nestling	26-May-2015	0.1791	7.0973	12
BKT2_5	BKT2	LOYAL	12	Nestling	26-May-2015	0.1791	7.0973	17
BKT2_6	BKT2	LOYAL	12	Nestling	25-May-2015	0.1791	7.0973	10
BKT2_7	BKT2	LOYAL	12	Nestling	25-May-2015	0.1791	7.0973	8
BKT2_8	BKT2	LOYAL	12	Nestling	25-May-2015	0.1791	7.0973	8
BKT2_9	BKT2	LOYAL	12	Nestling	25-May-2015	0.1791	7.0973	8
BKT3_1	BKT3	LAUREL	12	Nestling	27-May-2015	0.0724	5.8823	46
BKT3_10	BKT3	LAUREL	12	AdultFemale	25-May-2015	0.0724	5.8823	47
BKT3_11	BKT3	LAUREL	12	AdultMale	25-May-2015	0.0724	5.8823	44
BKT3_12	BKT3	LAUREL	12	Nestling	25-May-2015	0.0724	5.8823	66
BKT3_2	BKT3	LAUREL	12	Nestling	27-May-2015	0.0724	5.8823	47
BKT3_3	BKT3	LAUREL	12	Nestling	27-May-2015	0.0724	5.8823	29
BKT3_4	BKT3	LAUREL	12	Nestling	27-May-2015	0.0724	5.8823	40
BKT3_5	BKT3	LAUREL	12	Nestling	27-May-2015	0.0724	5.8823	24
BKT3_6	BKT3	LAUREL	12	Nestling	25-May-2015	0.0724	5.8823	25
BKT3_7	BKT3	LAUREL	12	Nestling	25-May-2015	0.0724	5.8823	25
BKT3_8	BKT3	LAUREL	12	Nestling	25-May-2015	0.0724	5.8823	34
BKT3_9	BKT3	LAUREL	12	Nestling	25-May-2015	0.0724	5.8823	44
BKT5_1	BKT5	POWD	17	Nestling	29-May-2015	0.1758	6.6661	11
BKT5_2	BKT5	POWD	17	Nestling	29-May-2015	0.1758	6.6661	17
BKT5_3	BKT5	POWD	17	Nestling	29-May-2015	0.1758	6.6661	31
BKT5_4	BKT5	POWD	17	Nestling	29-May-2015	0.1758	6.6661	24
BKT5_5	BKT5	POWD	17	Nestling	28-May-2015	0.1758	6.6661	29
BKT5_6	BKT5	POWD	17	Nestling	28-May-2015	0.1758	6.6661	23
BKT5_7	BKT5	POWD	17	Nestling	28-May-2015	0.1758	6.6661	21
BKT5_8	BKT5	POWD	17	Nestling	28-May-2015	0.1758	6.6661	45

SUPPLEMENTAL TABLE C.3. *Continued.*

SAMPLE_ID	NEST_ID	STREAM	FLAG	AGE_SEX	COLLECTION_DATE	PERCENT_EPT	AVG_PH	MOTU_RICHNESS
BKT5_9	BKT5	POWD	17	Nestling	28-May-2015	0.1758	6.6661	35
BKT7_1	BKT7	POWD	27	Nestling	26-May-2015	0.1628	6.6913	15
BKT7_10	BKT7	POWD	27	AdultMale	28-May-2015	0.1628	6.6913	26
BKT7_2	BKT7	POWD	27	Nestling	26-May-2015	0.1628	6.6913	14
BKT7_3	BKT7	POWD	27	Nestling	26-May-2015	0.1628	6.6913	29
BKT7_4	BKT7	POWD	27	Nestling	26-May-2015	0.1628	6.6913	32
BKT7_5	BKT7	POWD	27	Nestling	28-May-2015	0.1628	6.6913	23
BKT7_6	BKT7	POWD	27	Nestling	28-May-2015	0.1628	6.6913	35
BKT7_7	BKT7	POWD	27	Nestling	28-May-2015	0.1628	6.6913	32
BKT7_8	BKT7	POWD	27	Nestling	28-May-2015	0.1628	6.6913	23
BKT7_9	BKT7	POWD	27	AdultFemale	28-May-2015	0.1628	6.6913	41
BKT9_1	BKT9	POWD	48	Nestling	29-May-2015	0.2044	6.8477	24
BKT9_10	BKT9	POWD	48	AdultMale	1-Jun-2015	0.2044	6.8477	55
BKT9_2	BKT9	POWD	48	Nestling	29-May-2015	0.2044	6.8477	35
BKT9_3	BKT9	POWD	48	Nestling	29-May-2015	0.2044	6.8477	28
BKT9_4	BKT9	POWD	48	Nestling	29-May-2015	0.2044	6.8477	31
BKT9_5	BKT9	POWD	48	Nestling	1-Jun-2015	0.2044	6.8477	26
BKT9_6	BKT9	POWD	48	Nestling	1-Jun-2015	0.2044	6.8477	32
BKT9_7	BKT9	POWD	48	Nestling	1-Jun-2015	0.2044	6.8477	37
BKT9_8	BKT9	POWD	48	Nestling	1-Jun-2015	0.2044	6.8477	25
BKT9_9	BKT9	POWD	48	AdultFemale	1-Jun-2015	0.2044	6.8477	30
DAM_1_1	DAM1	POWD	8	Nestling	21-May-2015	0.1580	6.5776	41
DAM_1_2	DAM1	POWD	8	Nestling	21-May-2015	0.1580	6.5776	31
DAM_1_3	DAM1	POWD	8	Nestling	21-May-2015	0.1580	6.5776	28
DAM_1_4	DAM1	POWD	8	Nestling	22-May-2015	0.1580	6.5776	28
DAM_1_5	DAM1	POWD	8	AdultFemale	22-May-2015	0.1580	6.5776	32
DAM_1_6	DAM1	POWD	8	Nestling	22-May-2015	0.1580	6.5776	45
DAM_1_7	DAM1	POWD	8	AdultMale	22-May-2015	0.1580	6.5776	59
MMP_1_1	MMP1	POWD	2	Nestling	25-May-2015	0.2296	6.3421	28
MMP_1_2	MMP1	POWD	2	Nestling	25-May-2015	0.2296	6.3421	22
MMP_1_3	MMP1	POWD	2	Nestling	25-May-2015	0.2296	6.3421	27
MMP_1_4	MMP1	POWD	2	Nestling	25-May-2015	0.2296	6.3421	29
MMP_1_5	MMP1	POWD	2	Nestling	25-May-2015	0.2296	6.3421	20
MMP_2_11	MMP2	POWD	40	AdultFemale	28-May-2015	0.2140	6.8031	22
MMP_2_12	MMP2	POWD	40	AdultMale	28-May-2015	0.2140	6.8031	52
MMP_2_3	MMP2	POWD	40	Nestling	26-May-2015	0.2140	6.8031	29
MMP_2_4	MMP2	POWD	40	Nestling	26-May-2015	0.2140	6.8031	27
MMP_2_5	MMP2	POWD	40	Nestling	26-May-2015	0.2140	6.8031	28
MMP_2_6	MMP2	POWD	40	Nestling	28-May-2015	0.2140	6.8031	50
MMP_2_7	MMP2	POWD	40	Nestling	28-May-2015	0.2140	6.8031	37
MMP_2_8	MMP2	POWD	40	Nestling	28-May-2015	0.2140	6.8031	37
MMP_2_9	MMP2	POWD	40	Nestling	28-May-2015	0.2140	6.8031	34

SUPPLEMENTAL TABLE C.4. Louisiana Waterthrush nest metadata for Chapter 3.

NEST_ID	STREAM	FLAG	PERCENT_EPT	AVG_PH	AVG_PH	LEVINS	AVG_MOTU_RICHNESS	STDEV_MOTU_RICHNESS	TOTAL_MOTU_RICHNESS
BKT15_1	LOYAL	54	0.269485904	7.22	7.0441	0.200079051	24.88888889	9.426617162	97
BKT15_14	POWD	28_TRIB	0.082872928	4.65	4.6273	0.357549407	48.5	10.83644644	152
BKT15_2	LOYAL	12	0.179063361	7.18	7.0973	0.105770751	13.66666667	6.800735254	51
BKT15_3	LAUREL	12	0.072390572	6.04	5.8823	0.267588933	38	13.33333333	133
BKT15_5	POWD	17	0.175750834	6.73	6.6661	0.245652174	26.22222222	10.1214843	107
BKT15_7	POWD	27	0.162763466	6.8	6.6913	0.24173913	25.375	7.945124291	105
BKT15_9	POWD	48	0.204414587	6.92	6.8477	0.214268775	29.75	4.773438413	119
DAM15_1	POWD	8	0.15795207	6.67	6.5776	0.235019763	34.6	7.893034904	132
MMP15_1	POWD	2	0.229577465	6.54	6.3421	0.147628458	25.2	3.962322551	57
MMP15_2	POWD	40	0.213963964	6.89	6.8031	0.301422925	34.57142857	7.97615494	135

SUPPLEMENTAL TABLE C.5. Individual pH measurements from Louisiana Waterthrush territories in Chapter 3.

STREAM	FLAG	PH	NEST	STREAM	FLAG	PH
LOYAL	46	6.4	BKT9	POWD	47	6.51
LOYAL	46	7	BKT9	POWD	47	6.59
LOYAL	46	7.01	BKT9	POWD	47	6.66
LOYAL	46	7.19	BKT9	POWD	47	6.79
LOYAL	46	7.26	BKT9	POWD	47	6.96
LOYAL	46	7.28	BKT9	POWD	47	7.04
LOYAL	46	7.28	BKT9	POWD	47	7.08
LOYAL	46	7.37	BKT9	POWD	47	7.15
LOYAL	46	7.67	BKT9	POWD	47	7.19
LOYAL	46	7.72	BKT9	POWD	47	7.27
POWD	28_TRIB	4.52	DAM1	POWD	6	6.31
POWD	28_TRIB	4.77	DAM1	POWD	6	6.35
LOYAL	13	6.7	DAM1	POWD	6	6.5
LOYAL	13	6.9	DAM1	POWD	6	6.51
LOYAL	13	6.99	DAM1	POWD	6	6.53
LOYAL	13	7.04	DAM1	POWD	6	6.54
LOYAL	13	7.2	DAM1	POWD	6	6.71
LOYAL	13	7.21	DAM1	POWD	6	6.85
LOYAL	13	7.23	DAM1	POWD	6	6.89
LOYAL	13	7.33	DAM1	POWD	6	7.46
LOYAL	13	7.56	MMP1	POWD	1	5.9
LOYAL	13	7.65	MMP1	POWD	1	6.01
LAUREL	10	5.31	MMP1	POWD	1	6.15
LAUREL	10	5.61	MMP1	POWD	1	6.16
LAUREL	10	5.65	MMP1	POWD	1	6.47
LAUREL	10	6.02	MMP1	POWD	1	6.57
LAUREL	10	6.13	MMP1	POWD	1	6.59
LAUREL	10	6.16	MMP1	POWD	1	6.64
LAUREL	10	6.18	MMP1	POWD	1	6.79
LAUREL	10	6.26	MMP1	POWD	1	7.05
LAUREL	10	6.35	MMP1	POWD	1	7.66
LAUREL	10	6.38	MMP2	POWD	43	6.52
LAUREL	10	6.43	MMP2	POWD	43	6.59
POWD	16	6.39	MMP2	POWD	43	6.63
POWD	16	6.44	MMP2	POWD	43	6.66
POWD	16	6.47	MMP2	POWD	43	6.8
POWD	16	6.59	MMP2	POWD	43	6.81
POWD	16	6.68	MMP2	POWD	43	7.05
POWD	16	6.81	MMP2	POWD	43	7.14
POWD	16	6.85	MMP2	POWD	43	7.35
POWD	16	6.88	MMP2	POWD	43	7.38
POWD	16	7.08				
POWD	16	7.15				
POWD	30	6.41				
POWD	30	6.45				
POWD	30	6.48				
POWD	30	6.5				
POWD	30	6.75				
POWD	30	6.79				
POWD	30	6.94				
POWD	30	7.04				
POWD	30	7.22				
POWD	30	7.42				

SUPPLEMENTAL TABLE C.6. Sticky trap samples from Louisiana Waterthrush territories in Chapter 3.

NEST	STREAM	FLAG	BANK	SIDE	COLLECTION_DATE	TOTAL_INDIVIDUALS	TOTAL_EPT_INDIVIDUALS	PERCENT_EPT
BKT3	LAUREL	10	LAB	Back	14-May-2015	730	2	0.0027
BKT3	LAUREL	10	LAB	Back	29-May-2015	541	3	0.0055
BKT3	LAUREL	10	LAB	Back	9-Jun-2015	731	18	0.0246
BKT3	LAUREL	10	LAB	Back	24-Jun-2015	922	12	0.0130
BKT3	LAUREL	10	LAB	Back	7-Jul-2015	831	2	0.0024
BKT2	LOYAL	13	LAB	Back	20-May-2015	904	62	0.0686
BKT2	LOYAL	13	LAB	Back	3-Jun-2015	720	64	0.0889
BKT2	LOYAL	13	LAB	Back	17-Jun-2015	1085	52	0.0479
BKT2	LOYAL	13	LAB	Back	30-Jun-2015	782	6	0.0077
BKT2	LOYAL	13	LAB	Back	15-Jul-2015	749	1	0.0013
BKT1	LOYAL	50	LAB	Back	20-May-2015	1001	171	0.1708
BKT1	LOYAL	50	LAB	Back	3-Jun-2015	644	139	0.2158
BKT1	LOYAL	50	LAB	Back	17-Jun-2015	668	77	0.1153
BKT1	LOYAL	50	LAB	Back	30-Jun-2015	520	10	0.0192
BKT1	LOYAL	50	LAB	Back	15-Jul-2015	556	2	0.0036
MMP1	POWD	1	LAB	Back	13-May-2015	504	5	0.0099
MMP1	POWD	1	LAB	Back	29-May-2015	976	11	0.0113
MMP1	POWD	1	LAB	Back	9-Jun-2015	952	50	0.0525
MMP1	POWD	1	LAB	Back	24-Jun-2015	927	31	0.0334
MMP1	POWD	1	LAB	Back	7-Jul-2015	777	12	0.0154
DAM1	POWD	6	LAB	Back	19-May-2015	741	12	0.0162
DAM1	POWD	6	LAB	Back	3-Jun-2015	889	28	0.0315
DAM1	POWD	6	LAB	Back	17-Jun-2015	980	36	0.0367
DAM1	POWD	6	LAB	Back	30-Jun-2015	829	14	0.0169
DAM1	POWD	6	LAB	Back	15-Jul-2015	967	9	0.0093
BKT5	POWD	16	LAB	Back	19-May-2015	593	14	0.0236
BKT5	POWD	16	LAB	Back	3-Jun-2015	695	20	0.0288
BKT5	POWD	16	LAB	Back	17-Jun-2015	888	41	0.0462
BKT5	POWD	16	LAB	Back	30-Jun-2015	766	4	0.0052
BKT5	POWD	16	LAB	Back	15-Jul-2015	753	6	0.0080
BKT7	POWD	30	LAB	Back	13-May-2015	480	8	0.0167
BKT7	POWD	30	LAB	Back	29-May-2015	667	32	0.0480
BKT7	POWD	30	LAB	Back	9-Jun-2015	815	42	0.0515
BKT7	POWD	30	LAB	Back	24-Jun-2015	1007	28	0.0278
BKT7	POWD	30	LAB	Back	7-Jul-2015	923	5	0.0054
MMP2	POWD	40	LAB	Back	13-May-2015	484	5	0.0103
MMP2	POWD	40	LAB	Back	29-May-2015	678	57	0.0841
MMP2	POWD	40	LAB	Back	9-Jun-2015	1415	64	0.0452
MMP2	POWD	40	LAB	Back	24-Jun-2015	731	38	0.0520
MMP2	POWD	40	LAB	Back	7-Jul-2015	994	11	0.0111
BKT9	POWD	45	LAB	Back	19-May-2015	752	43	0.0572
BKT9	POWD	45	LAB	Back	3-Jun-2015	844	64	0.0758
BKT9	POWD	45	LAB	Back	17-Jun-2015	1479	99	0.0669
BKT9	POWD	45	LAB	Back	30-Jun-2015	1033	34	0.0329
BKT9	POWD	45	LAB	Back	15-Jul-2015	831	7	0.0084
BKT14	POWD	28_TRIB	LAB	Back	29-May-2015	606	1	0.0017
BKT14	POWD	28_TRIB	LAB	Back	3-Jun-2015	597	4	0.0067

SUPPLEMENTAL TABLE C.6. *Continued.*

Baetidae	Ephemeridae	Ephemerllidae	Heptageniidae	Leptophlebiidae	Ameletidae	Unknown_Ephemeroptera	Chloroperlidae
0	0	0	0	0	0	0	2
0	0	0	0	0	0	0	3
1	0	0	0	0	0	0	15
0	0	0	0	0	0	0	12
0	0	0	0	0	0	0	2
4	0	0	0	0	0	0	34
1	0	1	1	0	0	0	56
0	0	0	0	0	0	0	47
0	0	0	0	0	0	0	6
0	0	0	1	0	0	0	0
0	0	0	2	0	0	0	116
2	0	3	0	0	0	0	119
0	0	0	1	0	0	0	73
0	0	0	0	1	0	0	6
0	0	0	0	0	0	0	2
0	0	0	0	1	0	0	2
0	0	0	0	0	0	0	8
0	0	0	1	0	0	0	22
0	0	2	0	0	0	0	11
0	0	0	0	0	0	0	8
0	0	0	1	0	0	0	9
0	1	0	3	1	0	0	13
0	0	0	2	2	0	0	23
0	0	0	1	0	0	0	7
0	0	0	0	1	0	0	4
0	0	0	2	1	0	0	9
0	0	0	1	0	0	0	7
1	0	0	1	0	0	0	12
0	0	0	0	0	0	0	2
0	0	0	1	0	0	0	0
1	0	0	0	0	0	0	6
0	0	0	0	0	0	1	27
0	0	1	0	1	0	0	28
0	0	0	2	0	0	0	18
0	0	0	1	0	0	0	3
0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	47
0	0	0	2	0	0	0	57
0	0	1	0	1	0	0	25
0	0	0	1	0	0	0	8
0	0	0	1	1	0	1	29
0	0	1	2	0	0	0	54
0	0	0	0	0	0	0	91
0	0	0	0	0	0	0	17
0	0	0	0	1	0	0	1
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	2

SUPPLEMENTAL TABLE C.6. *Continued.*

Leuctridae (NOT INCLUDED)	Nemouridae NOT INCLUDED)	Peltoperlidae	Perlidae	Perlodidae	Beraeidae	Brachycentridae
27	0	0	0	0	0	0
11	0	0	0	0	0	0
2	4	1	0	0	0	0
20	1	0	0	0	0	0
47	2	0	0	0	0	0
0	3	0	0	21	0	0
0	5	0	0	0	0	0
2	0	0	0	0	1	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
2	4	0	0	36	0	0
2	1	0	0	5	4	0
0	1	0	0	1	0	0
1	0	0	0	1	1	0
5	0	0	0	0	0	0
2	1	0	0	2	0	0
3	0	0	0	2	0	1
0	0	1	0	1	23	0
3	1	2	0	2	10	0
2	0	1	1	0	1	0
10	0	0	0	1	1	0
4	0	2	0	2	2	2
3	0	1	0	4	3	0
0	1	2	1	2	0	0
0	0	0	0	4	0	0
6	0	1	0	0	0	0
3	1	1	0	0	5	1
4	1	11	0	0	3	0
1	1	0	0	0	0	0
3	2	0	0	4	0	0
21	0	0	0	1	0	0
1	1	0	0	2	0	0
7	3	3	0	0	5	0
6	3	1	0	1	2	0
1	0	0	0	1	0	0
20	1	0	0	1	0	0
2	1	3	0	1	0	0
2	3	0	0	0	2	0
5	6	0	0	3	0	2
3	0	0	0	1	0	0
10	0	2	0	3	3	0
3	3	1	0	2	0	0
7	8	1	0	2	1	0
2	3	0	0	0	0	2
24	0	0	0	1	0	0
25	4	0	0	0	0	0
32	0	0	0	0	0	0

SUPPLEMENTAL TABLE C.6. *Continued.*

Goeridae	Heliopsychidae	Hydropsychidae	Lepidostomatidae	Limnephilidae	Molannidae	Odontoceridae	Philopotamidae
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	3	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	12	0	0	0	0	0
1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
2	0	0	3	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
3	0	1	0	0	0	0	0
0	0	1	0	0	0	0	0
0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
1	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0
0	0	2	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	2

SUPPLEMENTAL TABLE C.6. *Continued.*

Phryganediae	Psychomyiidae	Rhyacophilidae	Unknown_Trichoptera
0	0	0	0
0	0	0	0
0	0	1	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	2	3
0	0	1	2
0	0	0	0
0	0	0	0
4	0	1	0
4	0	1	0
0	0	1	1
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
0	0	1	1
0	0	3	0
0	0	0	1
0	0	0	0
0	0	0	1
0	0	0	1
0	0	0	1
0	0	0	0
0	0	0	1
1	0	2	0
2	0	0	6
1	0	1	0
0	0	1	0
0	0	0	0
0	0	0	1
0	0	3	1
0	0	3	0
0	0	0	0
0	0	0	1
0	0	0	2
0	0	2	0
1	0	2	2
0	0	0	1
0	0	0	2
0	0	0	2
0	0	2	2
2	0	11	0
0	0	4	0
0	0	0	0
0	0	0	0

SUPPLEMENTAL TABLE C.7. Benthic samples from Louisiana Waterthrush territories in Chapter 3.

NEST_TERRITORY	STREAM	FLAG	DATE_COLLECTED	ENUMERATED_BY	SORTED_BY	SAMPLE_TYPE	PHYLUM	CLASS	ORDER	FAMILY	GENUS	QUANTITY
BKT3	LAUREL	10	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Crustacea	Decapoda	Cambaridae	Cambarus	2
BKT3	LAUREL	10	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Chironomidae	Unidentified	3
BKT3	LAUREL	10	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Tipulidae	Ormosia	1
BKT3	LAUREL	10	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Tipulidae	Tipula	1
BKT3	LAUREL	10	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Tipulidae	Dicranota	1
BKT3	LAUREL	10	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Tipulidae	Hexatoma	2
BKT3	LAUREL	10	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Baetidae	Baetis	28
BKT3	LAUREL	10	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Cinygmula	1
BKT3	LAUREL	10	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Crustacea	Isopoda	Asellidae	Caecidotea	2
BKT3	LAUREL	10	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Megaloptera	Corydalidae	Nigronia	1
BKT3	LAUREL	10	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Leuctridae	Leuctra	49
BKT3	LAUREL	10	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Nemouridae	Amphinemura	84
BKT3	LAUREL	10	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Peltoperlidae	Peltoperla	1
BKT3	LAUREL	10	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Perlidae	Acroneuria	1
BKT3	LAUREL	10	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Perlodidae	Isoperla	2
BKT3	LAUREL	10	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Hydropsychidae	Hydropsyche	1
BKT3	LAUREL	10	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Hydropsychidae	Diplectrona	2
BKT3	LAUREL	10	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Lepidostomatidae	Lepidostoma	4
BKT2	LOYAL	13	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Coleoptera	Elmidae	Stenelmis	1
BKT2	LOYAL	13	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Coleoptera	Elmidae	Oulimnius	20
BKT2	LOYAL	13	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Coleoptera	Elmidae	Optioservus	12
BKT2	LOYAL	13	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Coleoptera	Psephenidae	Ectopria	1
BKT2	LOYAL	13	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Chironomidae	Unidentified	16
BKT2	LOYAL	13	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Tipulidae	Dicranota	2
BKT2	LOYAL	13	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Baetidae	Baetis	68
BKT2	LOYAL	13	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Baetidae	Acentrella	18
BKT2	LOYAL	13	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Eurylophella	2
BKT2	LOYAL	13	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Ephemerella	14
BKT2	LOYAL	13	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Stenacron	1
BKT2	LOYAL	13	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	Paraleptophlebia	5
BKT2	LOYAL	13	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Chloroperlidae	Haploperla	4
BKT2	LOYAL	13	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Leuctridae	Leuctra	11
BKT2	LOYAL	13	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Perlidae	Acroneuria	2
BKT2	LOYAL	13	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Perlodidae	Isoperla	3
BKT2	LOYAL	13	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Taeniopterygidae	Taeniopteryx	3
BKT2	LOYAL	13	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Hydropsychidae	Hydropsyche	1
BKT2	LOYAL	13	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Hydropsychidae	Diplectrona	3
BKT2	LOYAL	13	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Hydroptilidae	Hydroptila	1
BKT2	LOYAL	13	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Philopotamidae	Dolophilodes	7
BKT2	LOYAL	13	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Polycentropodidae	Polycentropus	1
BKT1	LOYAL	50	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Coleoptera	Elmidae	Oulimnius	20
BKT1	LOYAL	50	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Coleoptera	Elmidae	Optioservus	17
BKT1	LOYAL	50	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Chironomidae	Unidentified	6
BKT1	LOYAL	50	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Empididae	Chelifera	3
BKT1	LOYAL	50	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Tipulidae	Dicranota	3
BKT1	LOYAL	50	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Baetidae	Plauditus	1
BKT1	LOYAL	50	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Baetidae	Baetis	44
BKT1	LOYAL	50	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Baetidae	Acentrella	57
BKT1	LOYAL	50	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Ephemerella	19
BKT1	LOYAL	50	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Isonychiidae	Isonychia	2
BKT1	LOYAL	50	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Chloroperlidae	Haploperla	3
BKT1	LOYAL	50	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Leuctridae	Leuctra	2
BKT1	LOYAL	50	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Nemouridae	Amphinemura	3
BKT1	LOYAL	50	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Perlidae	Acroneuria	3
BKT1	LOYAL	50	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Perlodidae	Isoperla	1
BKT1	LOYAL	50	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Taeniopterygidae	Taeniopteryx	3
BKT1	LOYAL	50	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Hydropsychidae	Diplectrona	1
BKT1	LOYAL	50	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Philopotamidae	Dolophilodes	19
BKT1	LOYAL	50	5/20/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Polycentropodidae	Polycentropus	1
BKT1	LOYAL	50	5/20/15	M. Logan	E.A.	Riffle	Annelida	Oligochaeta	Unidentified	Unidentified	Unidentified	1
BKT1	LOYAL	50	5/20/15	M. Logan	E.A.	Riffle	Mollusca	Bivalvia	Veneroida	Sphaeriidae	Pisidium	1
BKT14	POWD	28_TRIB	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Coleoptera	Elmidae	Oulimnius	1
BKT14	POWD	28_TRIB	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Crustacea	Decapoda	Cambaridae	Cambarus	2
BKT14	POWD	28_TRIB	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Ceratopogonidae	Probezzia	2
BKT14	POWD	28_TRIB	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Ceratopogonidae	Ceratopogon	1
BKT14	POWD	28_TRIB	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Chironomidae	Unidentified	36
BKT14	POWD	28_TRIB	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Simuliidae	Simulium	4
BKT14	POWD	28_TRIB	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Tipulidae	Limnophila	2
BKT14	POWD	28_TRIB	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Crustacea	Isopoda	Asellidae	Caecidotea	9
BKT14	POWD	28_TRIB	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Megaloptera	Corydalidae	Nigronia	1
BKT14	POWD	28_TRIB	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Leuctridae	Leuctra	52
BKT14	POWD	28_TRIB	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Nemouridae	Amphinemura	17
BKT14	POWD	28_TRIB	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Peltoperlidae	Peltoperla	40
BKT14	POWD	28_TRIB	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Hydropsychidae	Diplectrona	1
BKT14	POWD	28_TRIB	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Lepidostomatidae	Lepidostoma	2
BKT14	POWD	28_TRIB	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Philopotamidae	Wormaldia	4
BKT14	POWD	28_TRIB	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Polycentropodidae	Polycentropus	1
BKT14	POWD	28_TRIB	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Rhyacophilidae	Rhyacophila	8
BKT14	POWD	28_TRIB	6/3/15	M. Logan	E.A.	Riffle	Annelida	Oligochaeta	Unidentified	Unidentified	Unidentified	13
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Coleoptera	Elmidae	Oulimnius	33
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Coleoptera	Elmidae	Optioservus	3
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Crustacea	Decapoda	Cambaridae	Cambarus	1
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Chironomidae	Unidentified	6
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Simuliidae	Simulium	1

SUPPLEMENTAL TABLE C.7. *Continued.*

NEST_TERRITORY	STREAM	FLAG	DATE_COLLECTED	ENUMERATED_BY	SORTED_BY	SAMPLE_TYPE	PHYLUM	CLASS	ORDER	FAMILY	GENUS	QUANTITY
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Baetidae	Diphetor	5
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Baetidae	Plauditus	53
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Baetidae	Baetis	3
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Baetidae	Acentrella	17
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Serratella	1
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Ephemerella	8
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Drunella	3
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Maccaffertium	1
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Cinygmula	9
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Epeorus	11
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	Paraleptophlebia	9
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Megaloptera	Sialidae	Sialis	1
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Odonata	Gomphidae	Lanthus	3
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Chloroperlidae	Suwallia	4
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Chloroperlidae	Haploperla	2
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Chloroperlidae	Alloperla	2
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Leuctridae	Leuctra	4
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Nemouridae	Amphinemura	1
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Peltoperlidae	Peltoperla	1
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Perlidae	Atteneuria	3
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Pteronarcyidae	Pteronarcys	1
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Glossosomatidae	Agapetus	1
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Hydropsychidae	Hydropsyche	3
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Hydropsychidae	Ceratopsyche	1
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Hydropsychidae	Diplectrona	10
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Lepidostomatidae	Lepidostoma	2
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Philopotamidae	Dolophilodes	7
MMP1	POWD	1	5/13/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Polycentropodidae	Polycentropus	1
BKT5	POWD	16	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Coleoptera	Elmidae	Oulimnius	46
BKT5	POWD	16	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Coleoptera	Elmidae	Optioservus	2
BKT5	POWD	16	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Coleoptera	Psephenidae	Ectopria	1
BKT5	POWD	16	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Chironomidae	Unidentified	14
BKT5	POWD	16	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Simuliidae	Simulium	1
BKT5	POWD	16	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Tipulidae	Antocha	1
BKT5	POWD	16	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Tipulidae	Dicranota	1
BKT5	POWD	16	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Baetidae	Diphetor	2
BKT5	POWD	16	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Baetidae	Plauditus	58
BKT5	POWD	16	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Serratella	2
BKT5	POWD	16	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Ephemerella	9
BKT5	POWD	16	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Epeorus	6
BKT5	POWD	16	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	Paraleptophlebia	8
BKT5	POWD	16	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Chloroperlidae	Suwallia	2
BKT5	POWD	16	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Leuctridae	Leuctra	2
BKT5	POWD	16	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Nemouridae	Amphinemura	4
BKT5	POWD	16	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Peltoperlidae	Peltoperla	7
BKT5	POWD	16	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Perlidae	Acroneuria	4
BKT5	POWD	16	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Perlodidae	Isoperla	2
BKT5	POWD	16	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Pteronarcyidae	Pteronarcys	1
BKT5	POWD	16	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Glossosomatidae	Agapetus	1
BKT5	POWD	16	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Hydropsychidae	Hydropsyche	1
BKT5	POWD	16	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Hydropsychidae	Diplectrona	17
BKT5	POWD	16	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Lepidostomatidae	Lepidostoma	2
BKT5	POWD	16	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Philopotamidae	Dolophilodes	14
BKT5	POWD	16	6/3/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Rhyacophilidae	Rhyacophila	2
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Coleoptera	Elmidae	Oulimnius	45
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Coleoptera	Elmidae	Optioservus	1
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Coleoptera	Psephenidae	Ectopria	3
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Ceratopogonidae	Probezzia	1
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Chironomidae	Unidentified	21
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Simuliidae	Simulium	2
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Tipulidae	Hexatoma	7
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Baetidae	Diphetor	1
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Baetidae	Plauditus	63
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Baetidae	Acentrella	1
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Serratella	1
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Ephemerella	3
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Drunella	4
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Stenacron	1
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Maccaffertium	1
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Epeorus	8
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	Paraleptophlebia	2
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Chloroperlidae	Sweltsa	1
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Chloroperlidae	Suwallia	3
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Leuctridae	Leuctra	4
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Nemouridae	Amphinemura	3
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Peltoperlidae	Peltoperla	1
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Perlodidae	Malirekus	2
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Glossosomatidae	Agapetus	2
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Hydropsychidae	Diplectrona	1
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Lepidostomatidae	Lepidostoma	1
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Philopotamidae	Dolophilodes	10
BKT7	POWD	30	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Rhyacophilidae	Rhyacophila	1
MMP2	POWD	40	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Coleoptera	Elmidae	Oulimnius	53
MMP2	POWD	40	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Coleoptera	Elmidae	Optioservus	1

SUPPLEMENTAL TABLE C.7. *Continued.*

NEST_TERRITORY	STREAM	FLAG	DATE_COLLECTED	ENUMERATED_BY	SORTED_BY	SAMPLE_TYPE	PHYLUM	CLASS	ORDER	FAMILY	GENUS	QUANTITY
MMP2	POWD	40	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Chironomidae	Unidentified	20
MMP2	POWD	40	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Tipulidae	Tipula	1
MMP2	POWD	40	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Tipulidae	Dicranota	2
MMP2	POWD	40	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Baetidae	Plauditus	80
MMP2	POWD	40	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Baetidae	Baetis	3
MMP2	POWD	40	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Eurylophella	2
MMP2	POWD	40	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Ephemerella	3
MMP2	POWD	40	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Drunella	1
MMP2	POWD	40	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Cinygmula	3
MMP2	POWD	40	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Epeorus	12
MMP2	POWD	40	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	Paraleptophlebia	5
MMP2	POWD	40	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Chloroperlidae	Suwallia	5
MMP2	POWD	40	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Chloroperlidae	Haploperla	1
MMP2	POWD	40	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Leuctridae	Leuctra	4
MMP2	POWD	40	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Nemouridae	Amphinemura	1
MMP2	POWD	40	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Perlidae	Acroneuria	3
MMP2	POWD	40	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Perlodidae	Isoperla	1
MMP2	POWD	40	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Pteronarcyidae	Pteronarcys	1
MMP2	POWD	40	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Glossosomatidae	Agapetus	2
MMP2	POWD	40	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Philopotamidae	Dolophilodes	9
MMP2	POWD	40	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Polycentropodidae	Polycentropus	1
MMP2	POWD	40	5/29/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Rhyacophilidae	Rhyacophila	1
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Coleoptera	Elmidae	Oulimnius	18
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Coleoptera	Elmidae	Optioservus	2
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Coleoptera	Elmidae	Promoresia	2
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Coleoptera	Psephenidae	Ectopria	2
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Crustacea	Decapoda	Cambaridae	Cambarus	1
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Diptera	Ceratopogonidae	Ceratopogon	1
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Diptera	Chironomidae	Unidentified	11
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Diptera	Simuliidae	Simulium	6
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Diptera	Tipulidae	Dicranota	6
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Ephemeroptera	Baetidae	Diphetor	1
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Ephemeroptera	Baetidae	Plauditus	39
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Ephemerella	9
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Drunella	3
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Stenacron	1
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Epeorus	30
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	Paraleptophlebia	3
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	Unidentified	2
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Plecoptera	Chloroperlidae	Sweltsa	1
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Plecoptera	Chloroperlidae	Suwallia	12
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Plecoptera	Chloroperlidae	Haploperla	3
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Plecoptera	Leuctridae	Leuctra	3
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Plecoptera	Nemouridae	Amphinemura	3
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Plecoptera	Peltoperlidae	Peltoperla	6
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Plecoptera	Perlidae	Acroneuria	5
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Plecoptera	Perlodidae	Isoperla	4
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Plecoptera	Perlodidae	Yugus	1
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Plecoptera	Pteronarcyidae	Pteronarcys	6
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Trichoptera	Glossosomatidae	Agapetus	1
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Trichoptera	Hydropsychidae	Hydropsyche	1
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Trichoptera	Hydropsychidae	Diplectrona	3
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Trichoptera	Philopotamidae	Dolophilodes	15
BKT9	POWD	45	6/3/15	M. Logan	B.C.T.	Riffle	Arthropoda	Insecta	Trichoptera	Rhyacophilidae	Rhyacophila	3
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Coleoptera	Elmidae	Oulimnius	17
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Coleoptera	Elmidae	Optioservus	2
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Coleoptera	Psephenidae	Ectopria	1
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Crustacea	Decapoda	Cambaridae	Cambarus	3
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Chironomidae	Unidentified	9
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Empididae	Chelifera	1
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Tanyderidae	Protoplasa	1
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Tipulidae	Tipula	1
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Tipulidae	Antocha	2
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Tipulidae	Dicranota	2
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Diptera	Tipulidae	Hexatoma	3
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Baetidae	Diphetor	2
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Baetidae	Plauditus	52
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Baetidae	Baetis	6
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Baetidae	Acentrella	3
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Serratella	1
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Ephemerella	11
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Leucrocuta	4
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Cinygmula	4
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Epeorus	6
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	Paraleptophlebia	21
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Megaloptera	Corydalidae	Nigronia	1
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Odonata	Gomphidae	Lanthus	1
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Leuctridae	Leuctra	6
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Nemouridae	Amphinemura	3
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Peltoperlidae	Peltoperla	4
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Plecoptera	Pteronarcyidae	Pteronarcys	1
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Glossosomatidae	Agapetus	3
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Hydropsychidae	Diplectrona	6
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Lepidostomatidae	Lepidostoma	2

SUPPLEMENTAL TABLE C.7. *Continued.*

NEST_TERRITORY	STREAM	FLAG	DATE_COLLECTED	ENUMERATED_BY	SORTED_BY	SAMPLE_TYPE	PHYLUM	CLASS	ORDER	FAMILY	GENUS	QUANTITY
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Limnephilidae	Pycnopsyche	1
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Philopotamidae	Dolophilodes	3
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Polycentropodidae	Polycentropus	1
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Psychomyiidae	Lype	1
DAM1	POWD	6	5/19/15	M. Logan	E.A.	Riffle	Arthropoda	Insecta	Trichoptera	Rhyacophilidae	Rhyacophila	1

APPENDIX D

MOTU representative sequences for BOLD identification

SUPPLEMENTAL DATA D.1. MOTU representative sequences from Louisiana Waterthrush nestling fecal samples collected from Arkansas in Chapter 1.

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SUPPLEMENTAL DATA D.2. MOTU representative sequences from Louisiana Waterthrush nestling fecal samples collected from Pennsylvania in Chapter 1.

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SUPPLEMENTAL DATA D.3. MOTU representative sequences from riparian nestling fecal samples collected in Chapter 2.

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SUPPLEMENTAL DATA D.4. MOTU representative sequences from adult and nestling Louisiana Waterthrush fecal samples collected in Chapter 3.

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