Quantifying neonicotinoid concentrations in Missouri wetlands and the potential effects to the associated avian community.

Final Report

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Introduction: Advances in insecticide science in the late twentieth century led to the development of neonicotinoids (Jeschke and Nauen 2008). Neonicotinoids are a class of systemic insecticides that are markedly less toxic to vertebrate wildlife and humans than previous insecticides, and have thus supplanted these broadly toxic insecticides for use in the US (Jeschke et al. 2011). However, despite low toxicity to vertebrates, neonicotinoids may indirectly harm wildlife; specifically, non-target insects and organisms that rely on insects as a food source (Pisa et al. 2014; Morrissey et al. 2015). Indeed, a study in the Netherlands identified a strong negative correlation between population trends of insectivorous birds and concentrations of the neonicotinoid imidacloprid (Hallmann et al. 2014). While Hallman et al. (2014) did not establish a causal link between neonicotinoid concentrations and insectivorous birds, there are numerous factors considered as contributing to declines in avian populations in the Netherlands and around the world, including a disruption to trophic food webs (Benton et al 2002; Murphy 2003; Frenzel et al. 2015).

Bird populations that rely on grass and farmlands of Europe and North America have rapidly declined over the past thirty years (Benton et al. 2002; Sauer et al. 2017). Causes of such declines have been debated and are likely linked to multiple stressors. Conversion of grass and shrublands to monocrop agriculture has led to habitat loss. (Murphy 2003; Frenzel et al. 2015). In addition, granivorous birds in agricultural landscapes may be prone to ingesting pesticide laden seeds (Avery et al. 1997). However, observed population declines have been greatest among aerial insectivores and granivorous birds that rely on arthropods as a food source during certain life-history (e.g. nesting, brood rearing, etc.) events (Benton et al. 2002; Nebel et al. 2010). Negative population trends of birds reliant on farmland that depend on arthropods as a food source suggest a multi-stressor response due to a food-web disruption in agricultural landscapes, habitat loss, and potential direct toxicity of common use pesticides to avian species (Boatman et al. 2004).

Impacts of neonicotinoids to non-target aquatic emergent insects are of particular concern because of their importance in both aquatic and terrestrial food webs. Aquatic insect emergence represents an important transfer of energy from the aquatic to the terrestrial environment, as terrestrial predators nesting or foraging in riparian areas often rely on emergent insects as a food source, especially during the breeding season (Nakano and Murakami 2001, Paetzold et al. 2005, Epanchin et al. 2010). Neonicotinoid insecticides have been implicated in the decline of aquatic insects, emergent insects, and both their terrestrial and aquatic predators (Chagnon et al. 2014, Sánchez-Bayo et al. 2016). Not only do emergent aquatic insects supplement diets of aerial insectivores, they are of greater nutritional quality than terrestrial insects, specifically containing greater concentrations of polyunsaturated fatty acids (Martin-Creuzburg et al. 2016; Popova et al. 2017). If neonicotinoid concentrations in wetlands are impacting aquatic invertebrate populations in either the amount or timing of food energy availability by aquatic insect emergence, it could have implications for aerial insectivore food supply and nestling development.

We designed a field experiment to evaluate the potential impacts of direct application of neonicotinoid treated seed on the transfer of energy from aquatic to terrestrial ecosystems, using tree swallows and emergent aquatic insects in Missouri wetland ecosystems. Tree swallows have been used as a study organism in previous environmental contaminant studies because a) nesting tree swallows readily use human and agriculturally impacted areas b) tree swallows can be attracted to study sites through the use of artificial nest boxes c) both adult and fledgling tree swallows are strictly insectivorous, and d) adults have been found to preferentially select for insects of aquatic origin (McCarty 2001; Mengelkoch et al. 2004). Therefore, our objectives were to evaluate the relationship between tree swallow nest success and productivity at wetlands that have been treated with neonicotinoid treated seed during the previous spring growing season and compare these metrics to those observed in wetlands where neonicotinoid treated seed had not been planted. Further, at study wetlands, we quantified emergent insect communities as a potential causal mechanism for variation in tree swallow nesting success. We hypothesized that tree swallows nesting at previously treated wetlands would experience lower nesting efficiency due to the altered emergence of insects at critical time periods (e.g. nest site selection, egg laying, brood rearing) during the spring nesting season. Further we suspected nestling diet, as a measure of nestling feather stable carbon and nitrogen isotope content, to vary with neonicotinoid treatment and concentrations at study wetlands.

Methods: We established 100 tree swallow nest boxes (five per study wetland) along the perimeter of study wetlands in spring 2016, with all boxes located within 10 meters of standing water (Figure 1). During nest monitoring we recorded age of nesting female as second-year or after-second-year determined by plumage color (Hussell 1983). When each clutch was complete (as determined by no additional eggs on consecutive visits) we measured maximum length and breadth (mm) of each egg which were used to calculate egg volume. At each nest box visit, we used a digital caliper to measure tarsus length (mm), and a 25g Pesola spring scale to measure mass (Pesola scale company) for each nestling. Tarsus length was used to standardize body mass and calculate a body index because of uncertainty in estimating nestling age (Stauss et al. 2005; Nomi et al. 2015; Twining et al. 2016). A nestling's tarsus grows at a constant rate for the first 12 days at which time the tarsus has reached adult length, making tarsus length a suitable surrogate for age (Mccarty 2001). We used naturally occurring carbon and nitrogen isotope ratios to determine the prevalence of aquatic insects in tree swallow nestling diets. Stable carbon and nitrogen isotopes are useful in determining origin (aquatic vs terrestrial) and trophic position of prey items in consumer diets (Hyodo 2015). On the final nest visit we removed three to five contour feathers from each nestling remaining in the nest box for stable isotope diet analysis. At the same time, we collected terrestrial and aquatic insects from within each wetland using sweep nets. Reference insect material was collected for stable isotope analysis in order to compare ^{13}C and ^{15}N values found in contour feathers of fledglings with potential aquatic and terrestrial diet items. Finally, to quantify emergent aquatic insects available as a food source for tree swallows, we deployed two insect emergence traps at each wetland to measure available emergent insect abundance and biomass.

Statistical analyses for both tree swallow and emergent insects were completed using Generalized Linear Mixed Effect Regression (GLMER) models or Linear Mixed Effect

Regression (LMER) models. For each dependent variable we developed a set of five to eight candidate models, including a statistical null (intercept only) and a biological null (environmental covariates) model, which we then ranked using Akaike Information Criterion corrected for small sample size (AICc) (Barton 2016, Bates et al. 2017). If the statistical or biological null was the top model, or approximated the top model, we concluded insufficient evidence to support any of our alternate hypotheses represented by other *a priori* models.

Results and Discussion: We collected 56 sediment and 51 water samples from study wetlands across three sampling periods and had them analyzed for the presence of neonicotinoids (Figures 2&3). Across sampling periods, 50 to 55% of sediment samples contained at least one neonicotinoid active ingredient with an overall mean and maximum total (=summed neonicotinoids) concentrations of 0.36 μg/kg and 6.70 μg/kg, respectively. Neonicotinoids were detected more frequently in water samples than sediments, with at least one active ingredient present in 70 to 80% of water samples across the three sampling periods. However, mean total water concentrations were an order of magnitude lower than associated sediment ranging from 0.01 to 0.02 μg/L across all sampling periods (Figure 3).

Major flooding occurred across Missouri in spring and summer 2017, resulting in the loss of emergent insect collection traps at two Conservation Areas, Columbia Bottom and Four Rivers. Additionally, the lack of nesting tree swallows at Duck Creek and Otter Slough led us to remove insect traps 22 days after deployment. Despite flooding, we had 40 emergent insect traps operating at 20 wetlands for variable time periods resulting in a total of 1,719 trap days over 75 calendar days (April – July) to assess available tree swallow food resources. We collected, identified, and weighed 17,835 aquatic emergent individuals from 59 insect families spanning eight orders. Of the insect orders we collected, *Dipterans* (16,570 individuals) comprised 93% of emergent insects collected with the next most abundant being individuals from the order *Coleoptera* (3%). Within the order *Diptera*, *Chironomidae* were most prevalent (89% of individuals sampled) followed by *Ceratopogonidae* (10%) and *Dolichopodidae* (<1%).

Similar to emergent insect traps, some tree swallow nesting structures were destroyed by spring flood events, which delayed nesting at Columbia Bottoms and Four Rivers CAs; however, we replaced nest boxes and nesting did eventually resume at these sites. We observed no nesting attempts at Duck Creek, Otter Slough, and Grand Pass CAs, despite having recorded nesting activity the previous year. We observed 36 nesting attempts at the remaining 70 nest boxes, and ultimately monitored 35 of the nesting attempts; one nest was unmonitored due to an unusually late start date. Among monitored nests, clutch size ranged from 4 to 7 eggs laid with an overall mean of 5.7 eggs per nest. Hatching success (hatched at least one egg) was high (94%), with no observed predation through the monitoring period. Overall fledging success was 91% of nests fledging at least one nestling; of nests that fledged at least one nestling >96% of nestlings eventually successfully fledged.

Although the stable isotope $\delta^{13}C$ is typically used to differentiate between aquatic and terrestrial food sources, stable isotope signatures of reference aquatic and terrestrial insects did not differ for $\delta^{13}C$ (-28.2‰, -27.7‰, respectively), so no further modelling based on $\delta^{13}C$ was completed (Kato et al 2004; Paetzold and Tockner 2005). Because $\delta^{15}N$ differed between ecosystem sources in our system and replicate feather $\delta^{15}N$ samples (*n*=11) were adequately precise (mean overall difference of 0.35‰ ± 0.75‰); we used $\delta^{15}N$ for food source modeling. Therefore, we included $\delta^{15}N$ of a nestling's feather as a covariate for nestling body condition and found it to be a significant positive predictor for body mass. With the knowledge that greater

$\delta^{15}N$ indicated an increase in aquatic prey items and increased body indexes through the first two measurements, we used the same *a priori* candidate models as other tree swallow dependent variables to explain differences in nestling feather $\delta^{15}N$. We found that $\delta^{15}N$ did not differ and we did not have sufficient evidence to suggest neonicotinoid treatment or concentrations influenced nestling diet.

We found no evidence that neonicotinoid treatment had an effect on emerging insects in our sampled populations. Dipterans made up most of the sample and were important study organisms as they are both important food resources for tree swallows and are moderately sensitive to neonicotinoid insecticides (Quinney and Ankney 1985; Morrissey et al. 2015). As water neonicotinoid concentrations were more temporally dependent, potentially due to local agriculture activity, it is possible that we missed short-term pulse exposures of neonicotinoids in our limited sampling that may have influenced insects at both aquatic and emergent life stages (Mohr et al. 2012, Main et al. 2014). Additionally no definitive impacts of neonicotinoids were realized in nesting tree swallows. Adult and juvenile tree swallows responded to environmental and chemical variables differently, however no causal links were tied to either neonicotinoid treatment or concentration. Although we did not draw an explicit link between neonicotinoids and tree swallow nesting, there is insufficient information to conclude neonicotinoid seed treatments are safe for insectivorous birds. It is possible that neonicotinoids are detrimental either to avian species other than our study species, or during other life-history events such as autumn migration.

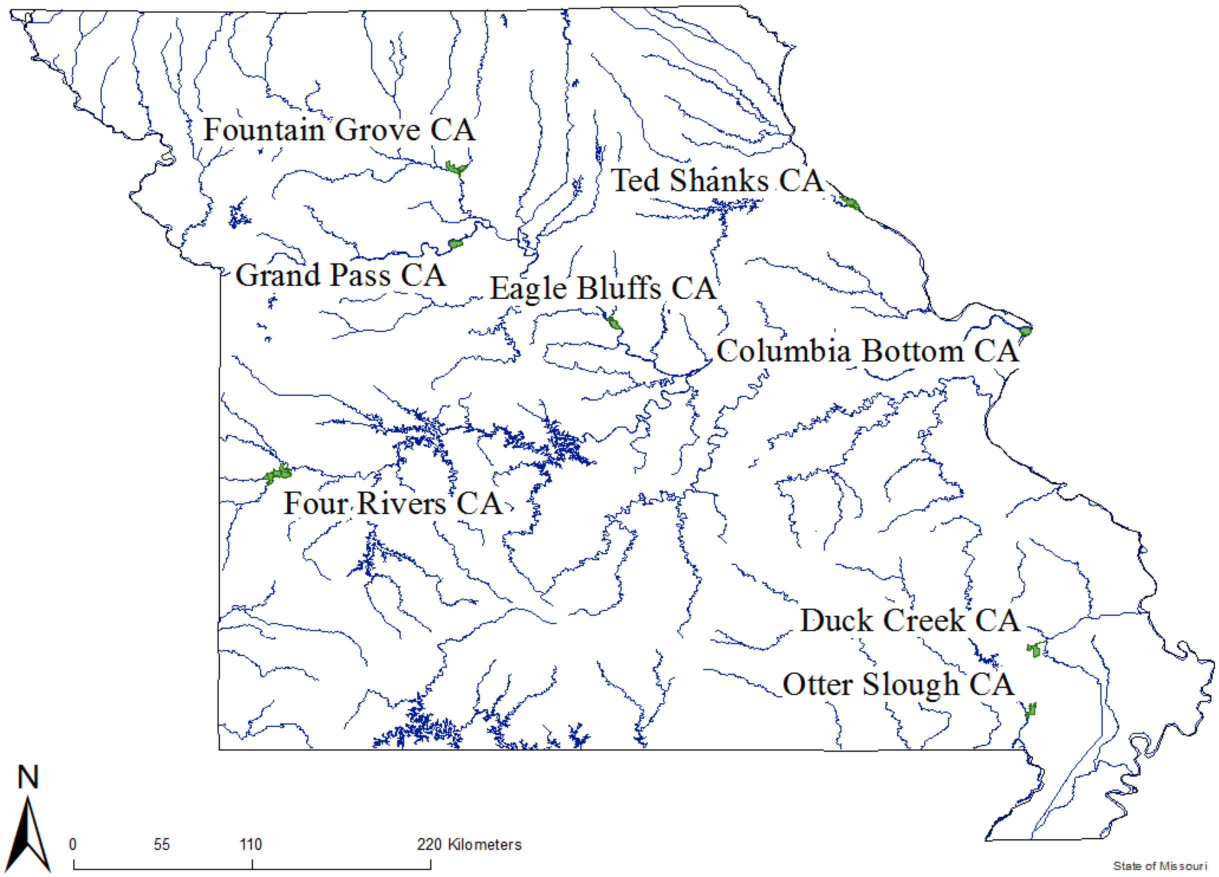

Figure 1: Study locations of Conservation Areas (CA) relative to major rivers and reservoirs in the state. Each study CA contained 2-4 study wetlands resulting in 20 total wetlands. Five tree-swallow nest boxes at each wetland (n = 100) were placed at study wetlands as well as emergent insect traps (n =40).

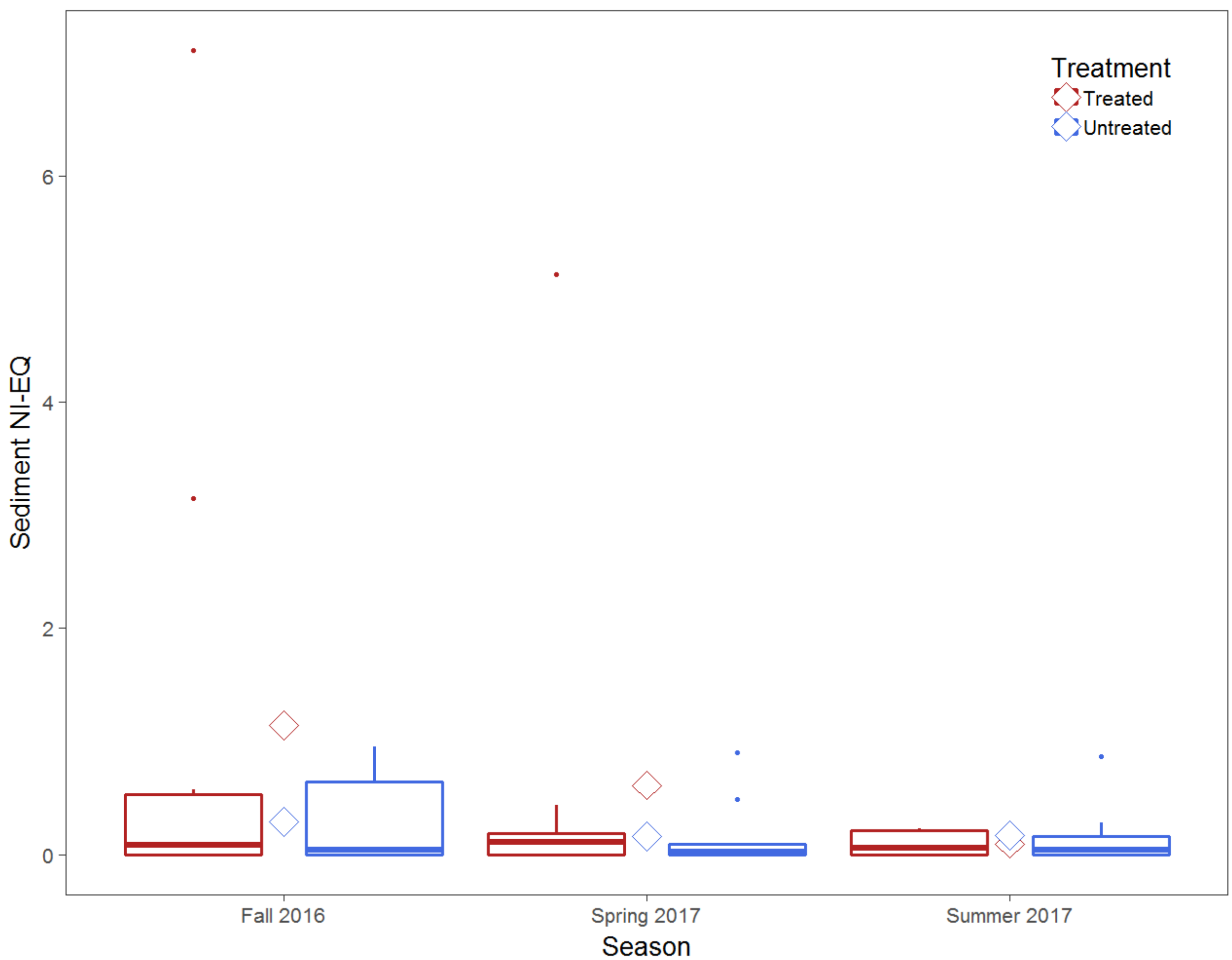

Figure 2: Sediment neonicotinoid toxic equivalencies (NI-EQs) for treated and untreated study wetlands across three sampling periods(Fall 2016, Spring 2017, and Summer 2017). Boxplots represent inter-quartile range of NI-EQs; points denote seasonal mean water NI-EQs.

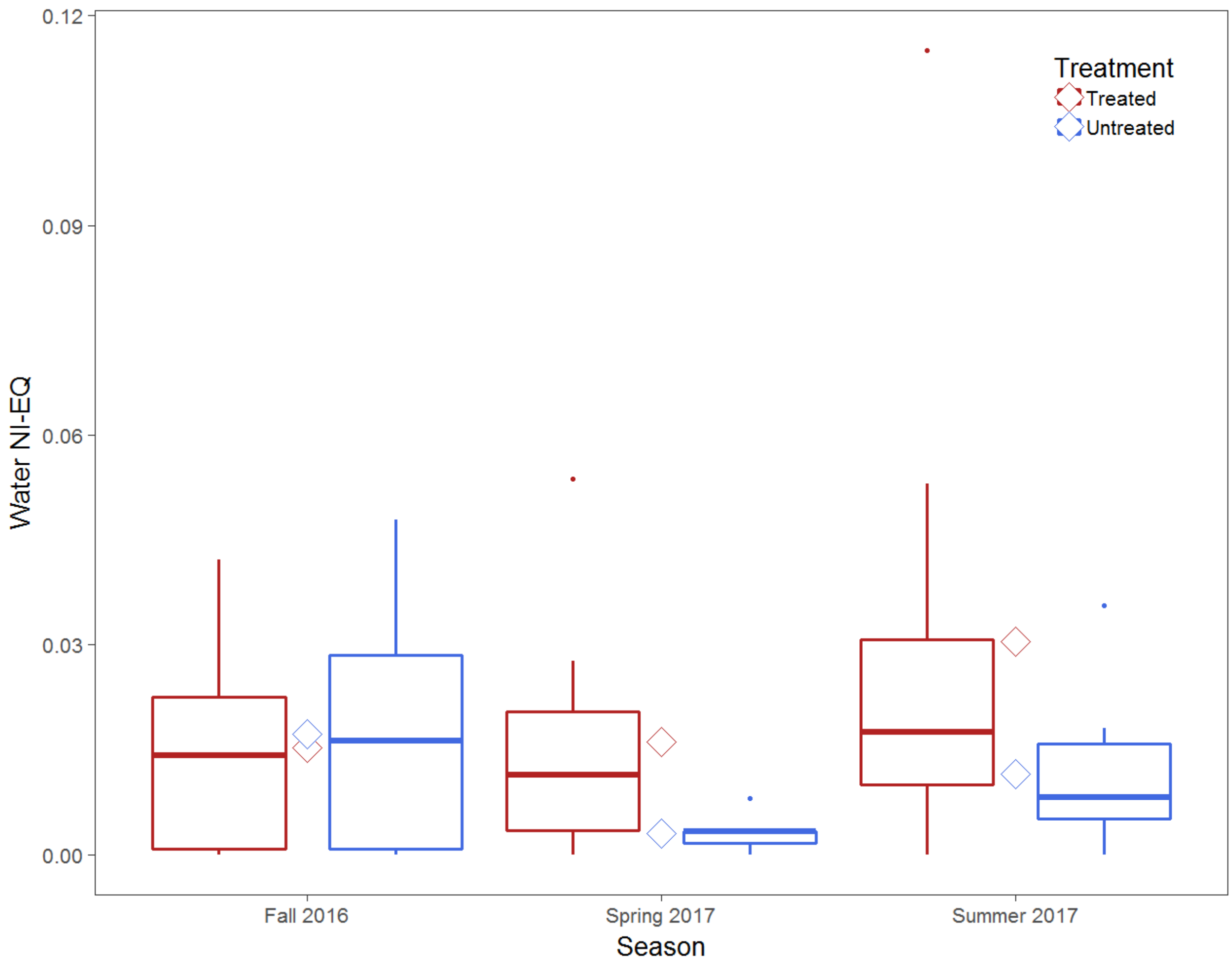

Figure 3: Water neonicotinoid toxic equivalencies (NI-EQs) for treated and untreated study wetlands across three sampling periods(Fall 2016, Spring 2017, and Summer 2017). Boxplots represent inter-quartile range of NI-EQs; points denote seasonal mean sediment NI-EQs.

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