NEONICOTINOIDS: ASSESSING THEIR DISTRIBUTION AND BIOLOGICAL EFFECTS TO PRAIRIE POTHOLE WETLANDS

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Title

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

The use of neonicotinoid pesticides is widespread throughout agricultural regions, including the Prairie Pothole Region (PPR) of North America. Recently, there have been growing concerns regarding the use of these pesticides and their potential impacts to non-target organisms, particularly honey bees and native pollinators. Neonicotinoids, being highly water soluble, have been found to occur widely in wetlands within the PPR, with potential impacts affecting sensitive aquatic insects. Prairie pothole wetlands are important ecological resources, producing over half of North America's duck populations. Using semi-field mesocosm experiments and a survey of PPR wetlands in Western Minnesota, I explored the distribution and concentration of neonicotinoids on the landscape and investigated the potential impacts to a group of aquatic insects belonging to the family Chironomidae. This research provides additional information on the fate of neonicotinoids on the landscape and their impact to sensitive aquatic insects.

ACKNOWLEDGEMENTS

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CHAPTER 1. INTRODUCTION

1.1. Neonicotinoids

1.1.1. Neonicotinoid Characteristics

Neonicotinoids, a group of systemic insecticides were first developed in the mid 1980's, and later introduced into the global market in 1991 with the first compound being imidacloprid. This group of insecticides are now licensed for use in more than 120 countries and are rapidly becoming one of the most widely used insecticide globally (Goulson 2013, Jeschke et al. 2010). Neonicotinoid compounds can be classified into three different chemical classes: N-nitroguanidies (imidacloprid, thiamethoxam, clothianidin and dinotefuran); nitromethylenes (nitenpyram) and N-cyanoamidiens (acetamiprid and thiacloprid)(Goulson 2013, Jeschke et al. 2010). The varying structures allows for versatility in the uptake and translocation of the compound throughout the plant. Their popularity can be attributed to their overall versatility through multiple application methods such as, irrigation, foliar spray and as well as through convenient pretreated seeds (Elbert et al. 2008, Jeschke et al. 2010) and are currently used in a variety of crops (Figure 1.1). The widespread adoption of neonicotinoid compounds can also be ascribed to their low toxicity to vertebrates and their high toxicity to most insect pests (Goulson 2013, Hladik et al. 2014, Tomizawa & Casida 2008). All neonicotinoids bind selectively to the nicotinic acetylcholine receptors in the invertebrates' nervous system causing both lethal and cumulative effects if exposed to repeated chronic levels of neonicotinoids (Morrissey et al. 2015). Despite their effectiveness to eliminate insect pests there is a growing concern that neonicotinoids are also causing adverse effects to non-target species particularly bees, pollinators, and aquatic invertebrates (Cavallaro et al. 2017, Krupke et al. 2012, Main et al. 2014, Rundlöf et al. 2015).

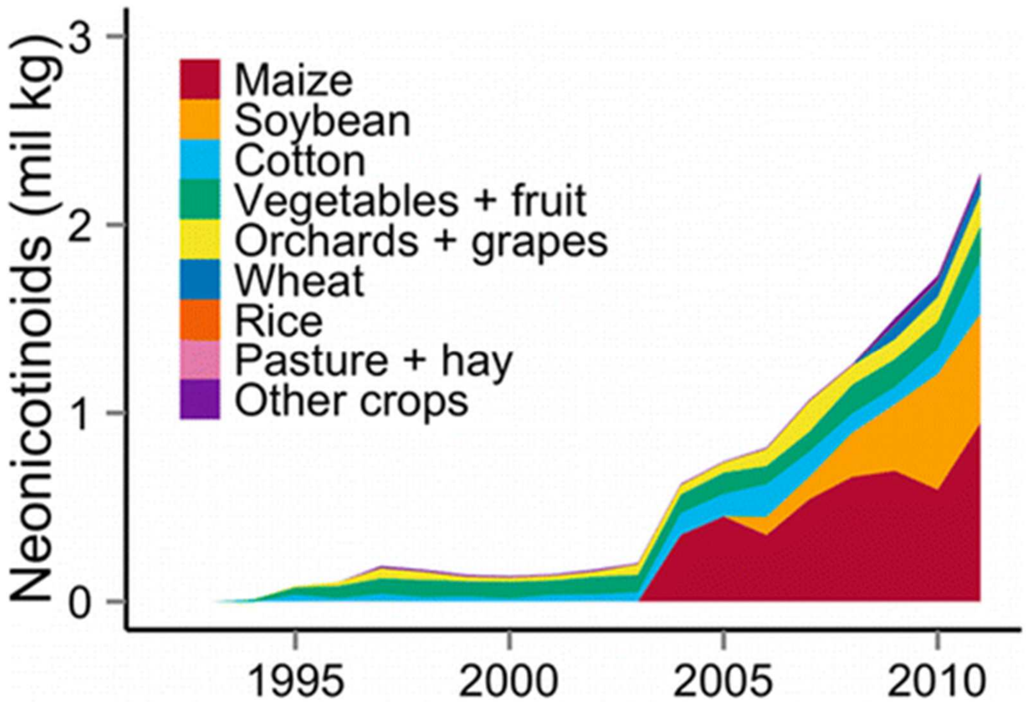

Figure 1.1. Neonicotinoid use among varying crop varieties and trends from 1992-2011 throughout the United States. (Reprinted with permission from Douglas and Tooker 2015 Copyright © 2015, American Chemical Society)

Aquatic systems may be impacted when surrounding upland landscapes are treated with neonicotinoid insecticides. Depending on the time of year and mode of application, neonicotinoids can accumulate in soils leaving up to 80% of the active ingredient remaining in the soil profile (Tapparo et al. 2012). With reported half-lives in excess of 1000 days, the potential contaminate can persist throughout multiple growing seasons (Goulson 2013). Neonicotinoid compounds as a class can be highly water soluble and, when paired with their persistence in soils, can have the potential to leach and move into both surface and groundwater systems (Hladik et al. 2014, Morrissey et al. 2015) Several studies have been conducted in both Europe (Van Dijk et al. 2013) as well as in parts of North America (Hladik et al. 2014, Main et al. 2014, Starner & Goh 2012) indicating widespread distribution of neonicotinoids in surface waters.

1.1.2. Neonicotinoids in Aquatic Ecosystems

The potential impacts on aquatic systems are of great concern in areas with high concentrations of wetlands coupled with high agricultural intensity, such as the Prairie Pothole Region (PPR). PPR wetlands are generally regarded as areas of high productivity and contain an abundance of plant and animal species. The PPR encompasses a 700,000 km^2 area extending from central Alberta to central Iowa (Figure 1.2), which includes nearly 6.5 million acres of wetlands (Dahl 2011) and is responsible for roughly 40-60% of the waterfowl production in North America (Guntenspergen et al. 2002). In a survey of wetlands in the Canadian prairies, (Main et al. 2014) found widespread use of neonicotinoids and reported detections of 94% of sampled areas. With approximately 80% of the water stored in these depressional wetlands coming from snowmelt and runoff, the likelihood of these basins becoming contaminated with neonicotinoids is highly likely (Main et al. 2016, Rickerl et al. 2000). The majority of wetlands in the PPR are often situated in areas with productive farmland where agricultural activities most often impact these basins either directly or indirectly (Kantrud et al. 1989). Understanding the potential impacts of pesticides on these small depressional wetlands is important, as these wetlands facilitate many key ecological processes, have high diversity, and aid in the detention of runoff and can reduce stream flow peaks and flooding (Rosen et al. 1995).

1.1.3. Neonicotinoid Impact to Aquatic Invertebrates

Chronic exposure of aquatic invertebrates to high concentrations of neonicotinoids may adversely affect growth, emergence, and behavior, which in turn can restructure aquatic invertebrate communities as well as the entire wetland ecosystem (Alexander et al. 2007, 2008, Morrissey et al. 2015). Aquatic invertebrates are a key component of the wetland ecosystem and can have important top-down effects that shape the distribution and abundance of macrophyte and algae communities (Wrubleski & Ross 2011). Further, aquatic invertebrates provide a vital food resource for waterfowl and other wetland fauna. For example (Swanson et al. 1985) found that during the months of June and July, aquatic invertebrates accounted for 89% of the diet of egg laying female mallards in south-central North Dakota. Thus, understanding the distribution of neonicotinoids on a local scale and investigating the potential impacts of neonicotinoids on aquatic invertebrate communities could provide beneficial information for guiding management decisions when assessing the impacts of neonicotinoid insecticides to PPR wetlands within the Midwestern States

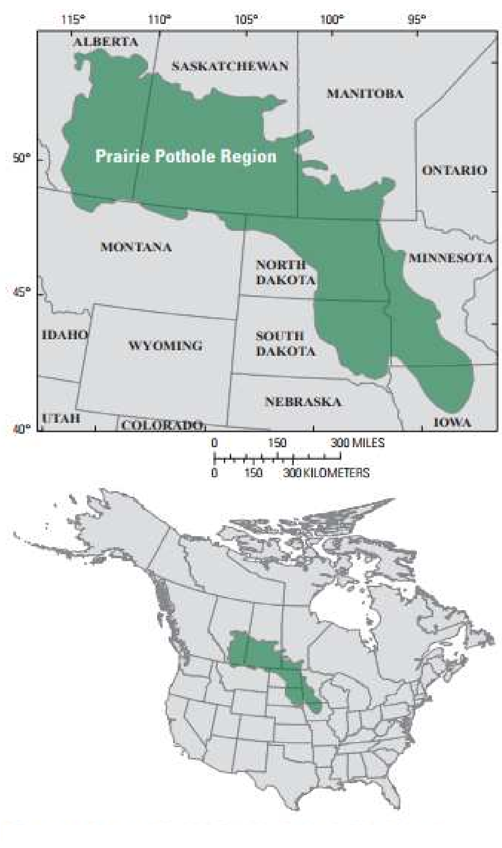

Figure 1.2. Map of Prairie Pothole Region. (from Renton et al. 2015)

1.2. Thesis Overview

This thesis consists of three chapters starting with a general introduction (Chapter One) followed by two additional chapters which consist of the results of original research conducted for this thesis. Both subsequent chapters have been written in a manuscript format for submission to peer-reviewed journals. Following the introductory chapter, the second chapter of this thesis, entitled "Effects of neonicotinoids on the emergence and composition of chironomids in the Prairie Pothole Region", focuses on the impacts of neonicotinoids on the members of the family Chironomidae. Mesocosm experiments were utilized to investigate how different concentrations of the neonicotinoid imidacloprid may impact community composition and emergence success. Results from these experiments indicate that neonicotinoids have the potential to reduce chironomid emergence and alter community composition. Chapter Three, entitled "Distribution and Concentration of Neonicotinoid Insecticides on Waterfowl Production Areas in West Central Minnesota", is an exploratory study of the distribution and concentration of neonicotinoids on the landscape in an effort to describe the extent to which neonicotinoids persist in regional wetlands, and understand potential threats to wetland ecosystems. This chapter describes the findings of a survey of 40 wetland sites conducted on waterfowl production areas throughout the PPR of West Central Minnesota. Results from this survey indicate that neonicotinoids are widely distributed throughout wetlands in west central Minnesota, at levels that have the potential to cause chronic impacts to sensitive aquatic insects. The conclusions provide additional information on the fate and potential biological impacts of neonicotinoids on regional wetlands, as researchers look to better understand the effects of neonicotinoids on aquatic ecosystems.

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CHAPTER 2. EFFECTS OF NEONICOTINOIDS ON THE EMERGENCE AND COMPOSITION OF CHIRONOMIDS IN THE PRAIRIE POTHOLE REGION

2.1. Abstract

The use of neonicotinoid pesticides is widespread throughout agricultural regions, including the Prairie Pothole Region of North America. Non target aquatic insects, such as chironomids have been shown to be particularly susceptible when exposed to compounds throughout their development and the reduction of these communities may have trophic level consequences within these systems. In the current study, field based mesocosms were utilized to investigate the effects of multiple pulses of the neonicotinoid imidacloprid on the emergence and chironomid community composition, in an effort to simulate episodic rain events to prairie pothole wetlands. Sediments from two nearby wetlands were placed into the mesocosm tanks and exposed to three pulses each one week apart at nominal concentrations of 0.2, 2.0 and 20 µg/L. Overall, a significant decrease in the emergence of adult chironomids were observed within the 2.0 µg/L and greater concentrations, with the subfamilies Chironominae and Tanypodinae showing a greater sensitivity than the members of the subfamily Orthocladiinae. The chironomid community also had a dose related response, followed by a recovery of the community composition near the end of the experiment. Our results provide additional evidence that repeated pulses of imidacloprid may have effects on chironomids and other sensitive aquatic insects living within Prairie Pothole wetlands, resulting in reduced food availability. We stress the need for continued monitoring of US surface waters for neonicotinoid compounds and the continuation of additional experiments looking into the impacts on aquatic communities.

[1] This material in this chapter was co-authored by Nathan Williams and Jon Sweetman. Nathan Williams had primary responsibility for collecting samples in the field, data analysis, and development of conclusions. Nathan Williams also drafted and revised all versions of this chapter. Jon Sweetman served as a proofreader and checked the math in the statistical analysis conducted by Nathan Williams.

2.2. Introduction

Increasing agricultural intensification is a significant threat to the quality of freshwater resources including an increased risk of chemical inputs from agricultural runoff (Stehle and Schulz 2015). The offsite transport of these compounds potentially raises concerns over the loss of sensitive aquatic communities, which are vital in maintaining ecosystem structure and provide valuable ecosystem services (Anderson et al. 2015). Aquatic invertebrates make up a large proportion of the biodiversity in freshwater food webs and provide a valuable link between primary producers and higher trophic level organisms (Chagnon et al. 2015). Growing reliance on the use of insecticides across the landscape potentially has unintended consequences, and understanding the potential impacts of these compounds is important for management.

Neonicotinoids are among the most popular and widely distributed insecticides currently in use (Jeschke et al. 2010; Van der Sluijs et al. 2013). These compounds act upon the nicotinic acetylcholine receptors (nAChRs) of insects and bind to the postsynaptic nAChRs, interfering with normal nervous system function, initiating paralysis and ultimately death (Tomizawa and Casida 2008). Neonicotinoids are applied to a range of crop types and can be delivered in a variety of mechanisms, including foliar applications, soil drenches and seed treatments. Among the application forms, seed treatments have received a considerable amount of attention (Douglas and Tooker 2015). Seed treatments are being applied prophylactically across the landscape and are estimated to account for approximately 60% of all applications (Jeschke et al. 2010). This method allows the active ingredient to be transported throughout the plant tissues, providing protection against pests during the plants early development (Elbert et al. 2008). While this application method mitigates some of the risk to bees and other pollinators, it has been shown that little of the active ingredient is taken up by the plant tissues, leaving the rest in the soil profile (Alford and Krupke 2017; Sur and Stork 2003). The high-water solubility of neonicotinoids and their persistence in soils has contributed to the offsite transport of these active ingredients to nearby waterbodies. Studies have documented detections of neonicotinoids in streams and rivers across the Midwestern United States (Hladik et al. 2014) as well as lentic systems including prairie pothole and playa wetland basins (Anderson et al. 2013; Main et al. 2014) found in Canada and the Southern United States.

The Prairie Pothole Region is a landscape particularly susceptible to the offsite transport of neonicotinoids. The region, known for its native prairie and millions of small wetland basis dispersed across the landscape, are becoming increasingly fragmented with the conversion of land use shifting towards large scale corn and soybean production (Wright and Wimberly 2013). Neonicotinoids have already been found to persist in wetlands within the Prairie Pothole Region, with multiple compounds being detected throughout the region (Main et al. 2014). Surface water samples collected from sites within cropped fields as well as areas within grassland vegetation all were positive for concentration of neonicotinoids with 90% of their spring water samples containing at least one compound (Main et al. 2014). The wetland habitats present in this area are key contributors in providing areas for biodiversity and habitat for many wetland dependent species.

Species such as waterfowl and insectivorous birds rely heavily on the abundance of aquatic insects, specifically chironomids to provide readily available food during important processes, such as brood rearing and migration (Bengtson 1972; Gray 1993). It has been shown that aquatic insects are more susceptible to the effects of neonicotinoids than other invertebrate's species (Morrissey et al. 2015). Chironomids (nonbiting midges) are ideal organisms to test, due to their ubiquity and abundance in aquatic ecosystems such as wetlands (Mousavi et al. 2003;

Saether 1979). The diversity and species richness of the family allows them to occupy a range of ecological niches and it has been shown that individuals can react differently to aquatic pollutants (Bazzanti and Bambacigno 1987).

To examine the potential impacts of neonicotinoids on the chironomid community composition in the Prairie Pothole Region we conducted a mesocosm experiment to investigate the effects of pulsed additions of imidacloprid (Merit 75 WP Insecticide 75% a.i) on the structure and emergence of adult chironomids within experimental mesocosms. Given the widespread distribution of neonicotinoids in aquatic systems, understanding their potential impacts on aquatic invertebrates is important for establishing thresholds that protect sensitive species. Single species laboratory tests are often used to observe the effects of a pesticide to a handful of standard aquatic species thus ignoring the effects to most of the other invertebrate species present in the community (Colombo et al. 2013; Pestana et al. 2009). The use of semi-field model ecosystems, known as mesocosms are a valuable tool that enable the opportunity to perform ecosystem-level research, by assessing contaminant effects on entire communities, while still allowing the statistical power through replication of multiple treatment types (Szöcs et al. 2015). By simulating the communities found within natural ponds and wetlands, the response from organisms exposed to a chemical disturbance should be more similar to a naturally occurring system (Touart and Slimak 1989) such as Prairie Pothole wetlands.

2.3. Methods

A series of outdoor aquatic mesocosm tanks located at the North Dakota Agricultural Experiment Station (NDAES), in Fargo, ND were set up to mimic conditions within the Prairie Pothole Region. Polyethylene cattle stock tanks (1211 L tanks; Ace Roto-Mold, Hospers, IA, USA) each filled with approximately 925 liters of municipal water, served as our experimental tanks. Before any water was transferred to an experimental tank, water was left to dechlorinate by evaporation for a minimum of 24 hours. To simulate natural invertebrate communities within our tanks we collected sediment and conducted sweep nets samples from two nearby local wetlands located within protected wildlife areas. Each tank was then inoculated with sediment to a uniform consistency of 5 centimeters throughout the bottom of each tank. Invertebrate samples from kick nets (500 μm mesh) from the two source wetlands were also evenly distributed among the tanks to provide species not found in the sediments. Following the addition of the sediments and the associated invertebrate community, the tanks were left undisturbed between 25 April 2016 and 23 May 2016 to allow all sediment to consolidate and to allow the invertebrate communities to establish.

Starting 26 May 2016 plexiglas floating conical emergent traps (diameter 63 cm) were placed on the water surface to collect emerging adult aquatic insects. An emergence sample from each mesocosm was collected every other day from 3 June 2016 until the end of the experiment on 9 August 2016. Emergent insects were preserved in a 50/50 solution of distilled water and 95% ethanol. Lab processing of invertebrates was initiated by emptying the contents of each sample into a 100 μm netting and then transferred to a separate dish. Using dissecting scopes, individuals were sorted, counted and identified to the lowest practical taxonomic level. Total counts were recorded for each taxonomic group.

To assess pre-impacted species composition among the tanks, we collected 6 samples prior to the initial insecticide treatment. On 14 June 2016, we exposed the mesocosms to one of four imidacloprid (Merit 75 WP Insecticide 75% a.i) concentrations (0, 0.2, 2, and 20 μg/L); these concentrations were based on a comprehensive species sensitivity distribution analysis of 214 toxicity tests completed by Morrissey et al. (2015). They proposed that any short-term peak neonicotinoid concentrations exceeding 0.2 μg/L could affect sensitive aquatic invertebrate populations. In addition to an untreated control we set our lowest concentration at 0.2 μg/l and increased by a magnitude of 10 for each level of 2 additional treatments; control, low, medium and high. Each treatment had 5 replicates for a total of 20 mesocosm tanks.

Since rainfall events can be a major driver in delivering pulses of neonicotinoids to nearby surface waters (Hladik and Kolpin 2015) two additional applications of imidacloprid were initiated a week apart, with the final application occurring 21 June 2016. Following each treatment of the insecticide one water sample from each treatment was tested for imidacloprid to ensure our dosage was correct. Water samples were priority shipped to the Montana State Analytical Lab. Water temperature, dissolved oxygen, conductivity and pH were measured daily in all tanks with a YSI meter (Professional Plus (Pro Plus) 6050000). In addition, temperature in each tank was recorded with individual data loggers throughout the experiment.

2.3.1. Data Analysis

To test for differences in the total overall abundance of chironomids (emerging adults) among the different treatment types, nonparametric methods were used due to non-normally distributed data. Kruskal-Wallis tests were performed and when a significant difference was detected pairwise Wilcoxon tests utilizing Dunnett's contrast with holm's correction were used to test which treatments were different from the controls. Alpha was set at 0.05 for all tests.

The effects of treatment and time on the Chironomidae community emerging throughout the experiment were analyzed using principal response curves (PRC), a multivariate method of analysis (Van den Brink and Ter Braak 1999). PRC's are a special form of redundancy analysis (RDA) that allow you to compare the compositional variation of the different treatment groups to the control groups through time (Van den Brink and Ter Braak 1999). Utilizing PRC, the resulting RDA axis of time is “partialled out” and the resulting axes displays the main interest in the analysis, the effects of the treatment and the interaction of time and treatment (Szöcs et al. 2015). Inclement weather caused the loss of 7 samples which accounted for 1% of the overall data. These samples were set as zeros and the abundance data were ln(2x+1) transformed before analysis. The significance of the overall treatment effect was tested using 1000 permutations to identify the dates for which treatments had a significant effect on the chironomid community. When permutation testing indicated no difference between control and treatment mesocosms for two consecutive sampling dates, it was determined that the community had recovered (Caquet et al. 2007).

All analyses were performed using R (R Core Team 2017). PRC’s were calculated using the vegan package (Oksanen et al. 2017) and restricted permutations were created using the permute package (Simpson 2016). Testing for differences between treatments was at the 5% significance level.

2.4. Results

2.4.1. Water Chemistry Parameters

The water of all mesocosms remained clear during the course of the experiment except for occasions at the start of the experiment, when macrophyte growth was minimal. Throughout the experiment, dissolved oxygen (DO) concentrations were not significantly different between experimental tanks, with mean values ranging from 78-81% among the different treatment types. Similarly, there were no significant differences in conductivity (range of 809-884 μS/cm during the course of the experiment) or pH (ranged of 9.35-9.53) (Table 1). We found no consistent significant effects of imidacloprid on the DO, pH or conductivity parameters (Dunnett’s test).

Table 2.1. Mean and standard deviation (Std.) of water chemistry parameters in experimental tanks 2016.

Parameters	**Unit**	**Treatments**							
		Control		**0.2 µg/L**		**2 µg/L**		**20 µg/L**	
		Mean	**Std.**	**Mean**	**Std.**	**Mean**	**Std.**	**Mean**	**Std.**
Temperature	°C	21.58	2.62	21.56	2.62	21.63	2.61	21.62	2.62
pH		9.35	0.46	9.53	0.47	9.47	0.39	9.53	0.45
DO	% Do	78.15	18.57	81.12	18.05	81.29	19.68	80.32	19.21
Conductivity	µS cm^{-1}	880.89	91.57	809.87	100.24	884.49	103.36	866.21	105.95

2.4.2. Emerging Chironomid Taxa

Over the experimental period, a total of 24 different genera of chironomids were identified in the mesocosms. The three most abundant taxa observed throughout the experiment included *Tanytarsus*, *Corynoneura* and *Procladius*. Other commonly found taxa included *Parachironomus, Chironomus and Cricotopus*.

2.4.3. Effects on Chironomid Community

Imidacloprid concentrations had significant effects on the overall abundance of emerging adult chironomids ($\chi^2 = 10.394$, $p > 0.01$). The average number of total chironomids emerging from the control tanks was 593.6 and decreased to 481.0, 256.6 and 195.8 among the low, medium, and high dosing concentrations, respectively (Figure 2.1). Post hoc multiple comparison tests indicated there were differences between the control and medium concentrations ($p=0.03$) as well as between the control and high dose concentration ($p < 0.02$). Sensitivity between different subfamilies was evident during the study. We found that members of the subfamily Orthocladiinae (*Corynoneura* sp. and *Cricotopus* sp.) were less sensitive to the treatments than other subfamilies and found no significant differences between the control and treatment concentrations (Figure 2.2).

PRC analyses of the Chironomidae community indicated that the three treatments (0.2, 2 and 20 ug/l) clearly deviated from the control community following the three sequential dosing events (Figure 2.3). See Table. 2.2 for a complete list of species weights and explained variance. The visual deviations of the treatments from the controls are consistent with the results of the permutation tests. Permutation testing indicated that the PRC diagram did display a significant proportion of variation of the first axis ($P < 0.01$). Of the total variance, 32.2% and 14.8% were attributed to the sampling date and treatments, respectively (Figure 2.3). 40.5 % of the explained variance is expressed by the first axis of the RDA and is displayed on the vertical axis (Table 2.2). Testing each sampling day indicated a treatment effect, days 4-8, followed by a slight recovery during sample days 10 and 12. However, after the final treatment, which occurred 14 days following the initial treatment, we saw a treatment effect persist from days 14-34 and indicated a recovery by day 38. The largest negative effects were observed for the genera *Tanytarsus*. There were no genera that showed a positive response following the different dose concentrations (Figure 2.3). Following treatments of the 2 and 20 µg/L doses we observed very few individuals emerging from these mesocosm tanks.

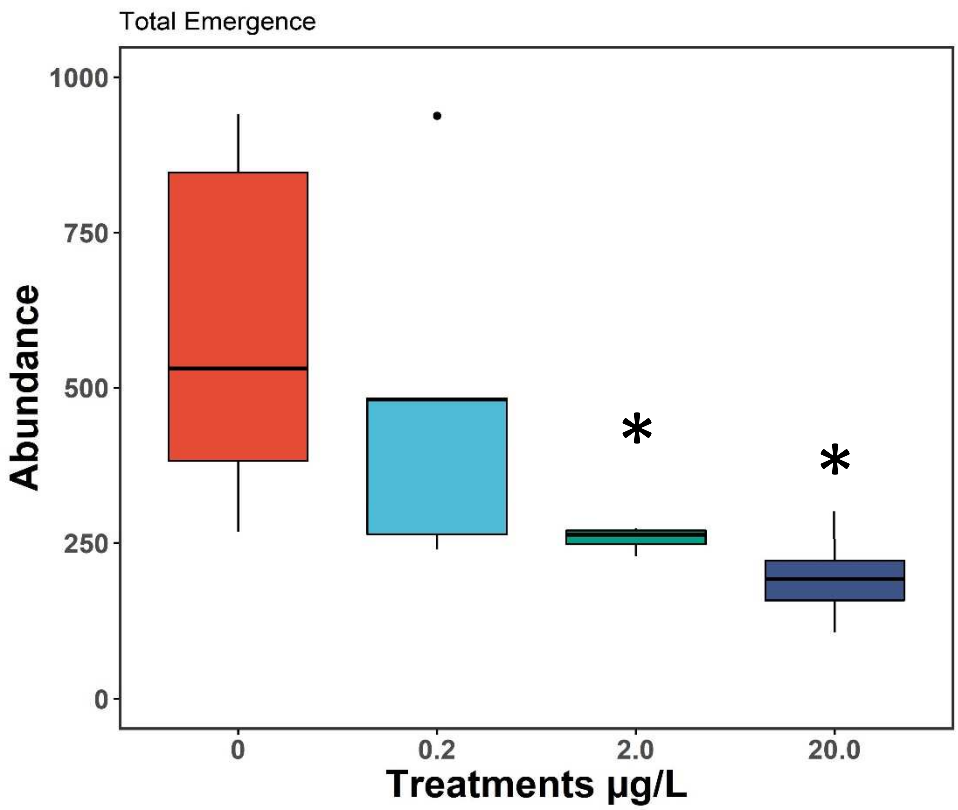

Figure 2.1. Comparison of total chironomid abundance across imidacloprid concentrations. *Treatments significantly different from controls ($p < 0.05$) Dots represent outliers.

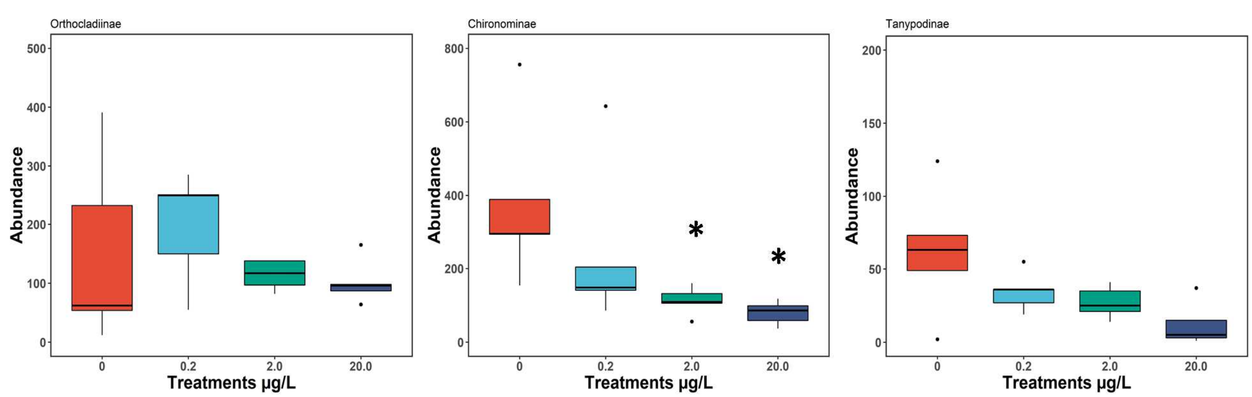

Figure 2.2. Comparison of total chironomid abundance across three different subfamilies subjected to imidacloprid additions. * Treatments significantly different from controls ($p < 0.05$) Dots represent outliers.

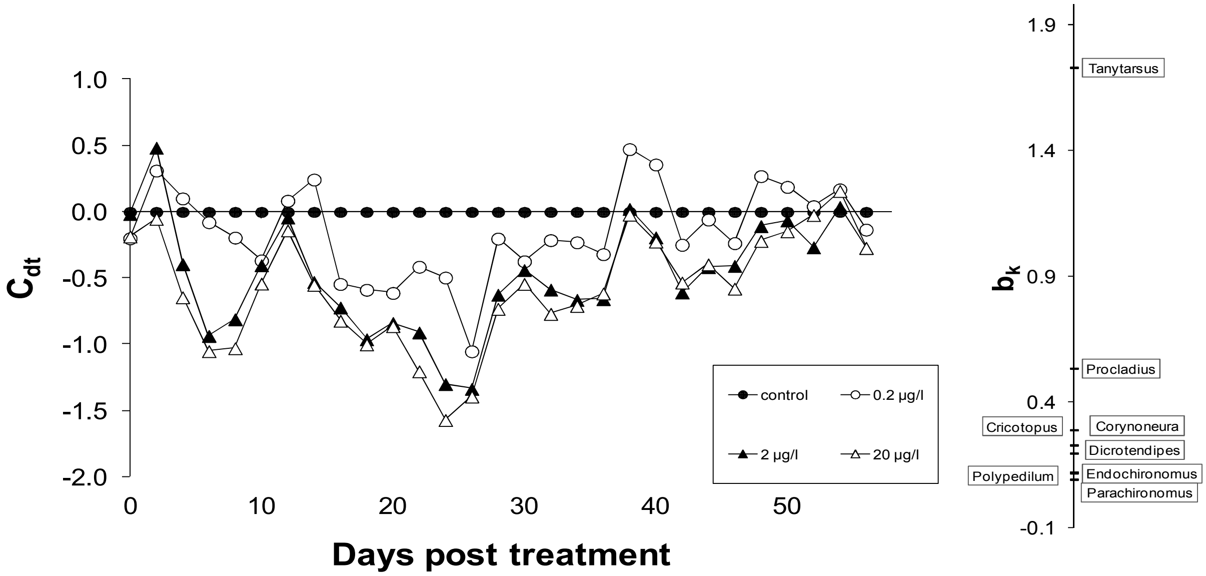

Figure 2.3. Principal response curve (PRC) diagram with species weights for the chironomid community, indicating the effects of multiple pulses of the insecticide imidacloprid. For percentages of variance accounted for and list of all the species weights see Table 2.2. The species weight (bk) can be interpreted as the relationship of the individual taxon to the PRC's.

Table 2.2. Percentages of the total variance that can be attributed to the time and treatment regime for the analyzed chironomid data, as well as the weight of the species scores of the different genera. The table also indicates the fraction of the variance that is explained by the treatment, captured on the first PRC curve.

Species	b_k-score
Chironomus	-0.0318
Pseudochironomus	-0.0039
Chaoborus	-0.0021
Psectrocladius	-0.0006
Psectrotanypus	-0.0003
Lauterborniella	0.0013
Limnophyes	0.0046
Cryptotendipes	0.0074
Paratendipes	0.0124
Micropsectra	0.0208
Ablabesmyia	0.0409
Cladopelma	0.0427
Glyptotendipes	0.0503
Cryptochironomus	0.0524
Labrundinia	0.0711
Polypedilum	0.0913
Endochironomus	0.1170
Parachironomus	0.1204
Dicrotendipes	0.1953
Corynoneura	0.2275
Cricotopus	0.2882
Procladius	0.5329
Tanytarsus	1.7284
% variance explained by	
Time	32
Treatment	15
first PRC	40

2.5. Discussion

Aquatic invertebrates, particularly insects, have been found to be highly sensitive to neonicotinoids. Previous literature reviews have indicated that imidacloprid is one of the most toxic neonicotinoids among the active ingredients on the market today (Morrissey et al. 2015). The Chironomidae community that was established throughout the mesocosm tanks was affected by the repeated pulses of a commercial formulation containing the active ingredient imidacloprid. Total adult emergence was decreased throughout the, 2.0 and 20 μg/L nominal concentrations. This result is comparable with previous studies, which showed macroinvertebrates response to repeated pulses of the compound imidacloprid (Cavallaro et al. 2017; Colombo et al. 2013; Mohr et al. 2012). For example, Cavallaro et al. 2017 found a decrease in emergence success of *Chironomus dilutus* following exposure to treatments of imidacloprid and observed excessive movement of larvae along the substrate surface as well as individuals becoming entangled to the pupal exuvia when attempting to emerge.

Members of the family Chironomidae are among the most sensitive taxa to neonicotinoids, with only species of Ephemeroptera and Trichoptera appearing to be one of the few taxa that are more sensitive to the compounds (Morrissey et al. 2015; Roessink et al. 2013). This study showed that adverse effects on the Chironomidae community composition may occur if repeated pulses, via snowmelt transport (Main et al. 2016) or rainfall events (Hladik and Kolpin 2015), of the active ingredient imidacloprid are introduced into wetlands across areas such as the Prairie Pothole Region. Many different factors can contribute to the overall sensitivity of an individual when exposed to a toxicant. Life history traits, including an individual's mobility potential, reproductive generations per year and the specific environmental conditions in which they develop can all play a key role in how a species will react (Cavallaro et al. 2017; Liess and Beketov 2011). Of the common taxa found within the mesocosm tanks the two most abundant taxa (*Tanytarsus* and *Corynoneura*) differed in their apparent sensitivity to imidacloprid pulses. Orthocladiinae which encompass genera such as *Corynoneura* and *Cricotopus* were found to be less sensitive during the experiment which could be a characteristic of the subfamily in which more species are known to be multivoltine, in which species are capable of producing multiple generations of offspring throughout the year (Tokeshi 1995). Since the mesocosm tanks were left open to the environment, emerging adults from the surround areas as well as experimental tanks could potentially oviposit eggs into any of the experimental tanks, which could also explain the recovery of the community following the treatment doses.

Insecticide presence in surface waters has been shown to impact water quality parameters (Kreutzweiser et al. 2002). For example, reductions in the structure of specific communities can cause changes in parameters such as dissolved oxygen. Kreutzweiser et al. (2002) found a significant concentration dependent increase in dissolved oxygen levels among mesocosms treated with an insecticide, suggesting that reductions of zooplankton populations may have caused a decrease in oxygen consumption by respiration. Imidacloprid has also been linked to changes within rice paddy fields, where researchers observed lower turbidity and higher dissolved oxygen most likely from the loss of aquatic invertebrates such ostracoda and chironomidae larvae, who are found near the sediments of the rice paddy fields (Hayasaka et al. 2012; Sánchez-Bayo and Goka 2006). In the present study, there were no indications that imidacloprid pulses had any impact on dissolved oxygen, pH, or specific conductivity among the treated mesocosms. The averages among the parameters were all within a similar range throughout the experiment. However, anecdotally, we did observe an increase in the number of snails populating the mesocosms treated with 20 µg/l imidacloprid and all mesocosm maintained a clear state.

The present mesocosm study provides a good example of the potential consequences of the offsite transport of neonicotinoid compounds such as imidacloprid and the effects they could have on aquatic arthropods such as Chironomids. Since natural populations and communities of aquatic organisms are often exposed to multiple pulses of an insecticide the use of three separate pulses provides a realistic exposure scenario in wetlands of agro-ecosystems such as the Prairie Pothole Region. On the landscape it is not uncommon for multiple basins to be exposed to the presence of a variety of agro-chemicals, emphasizing the need for continued research on the effects of multiple stressors and the importance of protected areas in providing refugia for sensitive species to have the ability to reproduce and recolonize impacted wetlands. However, it is important to keep in mind that not all species found within these areas have the ease of mobility and can produce multiple generations of offspring each year. Consequently, more tolerant species may become the dominant taxa throughout an ecosystem, (Columbo et al. 2013) leading to an imbalance among the aquatic food web which could cause cascading trophic level effects.

2.6. Conclusion

This study highlighted the effects of the neonicotinoid, imidacloprid on a chironomid community exposed to a series of short term pulses. Increased use of neonicotinoids and other pesticides is common in areas such as the Prairie Pothole Region putting millions of small wetland basins at risk. Since the primary exposure scenario often occurs during rainfall events it is not rare for many of these individuals to be exposed on a recurring basis. Direct effects, as shown in this study, through decreased emergence, as well as indirect effects can all have implications for the aquatic food web. The use of field relevant concentration within mesocosms provides an ideal testing scenario that enables researchers to examine the effects of contaminants in a semi-realistic environment.

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CHAPTER 3. DISTRIBUTION AND CONCENTRATION OF NEONICOTINOID INSECTICIDES ON WATERFOWL PRODUCTION AREAS IN WEST CENTRAL MINNESOTA

3.1. Abstract

Neonicotinoid insecticides have been reported to occur widely in surface waters, including wetlands within the Prairie Pothole Region (PPR). In the US portion of the PPR, the US Fish and Wildlife Service has established Waterfowl Production Areas (WPAs) in an effort to enhance waterfowl production within the region. Most WPAs have an area of protected upland surrounding the wetland, which may act as a buffer to limit runoff and accumulation of pesticides. However, the intensity of agricultural activity varies greatly around such buffers. We assessed the extent that neonicotinoid insecticides occurred in wetlands within WPAs throughout west central Minnesota based on a gradient of agricultural activity. Of the five neonicotinoids measured, at least one of the three most commonly occurring compounds, imidacloprid, clothianidin or thiamethoxam were detected in 29% of our wetland water samples, and both detections and total concentration of neonicotinoids were higher in sites with higher surrounding crop use. Neonicotinoid insecticides if persistent for long periods of time have the potential to affect sensitive aquatic invertebrate communities within prairie wetlands. Our research indicates that areas perceived as protected may still be at risk to the offsite transport of neonicotinoids, emphasizing the importance of maintaining effective grassland buffers around wetlands found in the prairies.

[1] This material in this chapter was co-authored by Nathan Williams and Jon Sweetman. Nathan Williams had primary responsibility for collecting samples in the field, data analysis, and development of conclusions. Nathan Williams also drafted and revised all versions of this chapter. Jon Sweetman served as a proofreader and checked the math in the statistical analysis conducted by Nathan Williams.

3.2. Introduction

Anthropogenic stressors to aquatic environments, including inputs from agrochemicals, can have detrimental impacts to these important resources. Wetlands provide areas of biodiversity and contribute vital ecological functions, for example, through groundwater recharge and the provisioning of food resources and habitat for a wide range of fish and wildlife species (Erwin 2009, Houlahan et al. 2006). While wetlands can be some of the most productive ecosystems in the world, the continued loss and deterioration of these habitats is accelerating in areas in demand for agricultural production, and the increased reliance on chemical fertilizers and pesticides has contributed to a growing concern about potential environmental impacts, including effects on aquatic ecosystems (McLaughlin &Mineau 1995). The contamination of insecticides to wetland waters could potentially affect the many non-target organisms, such as aquatic insects, and consequently waterfowl, fish and other organisms that rely on freshwater invertebrates through cascading effects throughout the food web.

Insecticide impacts are of particular interest for the Prairie Pothole Region of North America, which is responsible for up to 60% of the waterfowl production in North America and provides critical habitat for many other wetland dependent species (Guntenspergen et al. 2002).The Prairie Pothole Region (PPR) encompasses an estimated 700,000 km^2 area, extending from central Alberta to central Iowa containing nearly 6.5 million acres of wetlands (Dahl 2011), which are embedded in significant agricultural landscape. Over the last decade or so this area has seen a significant change in land-use practices, with many farms shifting towards large scale operations, relying heavily on the use of insecticides to limit crop damage and improve agricultural yields (Meehan et al. 2011).

The reliance on insecticide use can be partially attributed to the introduction and rapid adoption of neonicotinoid insecticides. This class of insecticide is one of the most widely used globally and accounts for nearly 26% of the global insecticide market (Sparks 2013). First developed in the 1980's and brought to market in the early 1990's, neonicotinoids are now licensed for use in over 120 countries worldwide. Valued for their versatility and broad spectrum toxicity, neonicotinoids are most commonly used as seed treatments, occurring on approximately 60% of all coated seeds (Cox Jr et al. 1998, Douglas &Tooker 2015, Jeschke et al. 2010). Facilitated by the high water solubility of neonicotinoids, the compounds are systemically taken up into the plant tissues, providing protection to the young germinating plant (Simon-Delso et al. 2015). However, studies have shown that less than 20% of the active ingredient may be taken up by the plant, with the rest potentially persisting in the surrounding soils (Sur &Stork 2003). With relatively long half-lives in soil, ranging in excess of 1000 days and its high water solubility there is the potential for these compounds to persist in the environment and be transported to surrounding water bodies via groundwater or surface runoff (Goulson 2013, Van Dijk et al. 2013).

With concern for the persistence and potential impact to the environment, there has been increasing interest in examining the fate and distribution of neonicotinoids across the landscape. Recent studies have shown that aquatic systems situated in agricultural regions are susceptible to contamination by neonicotinoid insecticides (Anderson et al. 2013, Hladik et al. 2014, Main et al. 2014). Concentrations of these compounds have all been found to occur in rivers, streams, lakes and wetlands receiving surface water from agricultural fields. Previous work from the prairies of Canada have shown that isolated basins devoid of buffer vegetation are of higher probability to become exposed to concentrations of neonicotinoids (Main et al. 2015). While wetlands located directly in agricultural fields within the Prairie Pothole Region have been shown to contain neonicotinoids (Evelsizer &Skopec 2016, Main et al. 2014), the levels of of contamination in more protected areas has not been evaluated. The purpose of this study is to describe the distribution and concentration of five common neonicotinoids in wetlands found on Waterfowl Production Areas (WPA's) in West Central Minnesota. WPA's managed by the United States Fish and Wildlife Service (USFWS), provide important waterfowl brood rearing habitat and serve as protection areas containing a mixture of grassland and wetland habitats. This study will provide additional insight into the fate and distribution of neonicotinoid compounds across the landscape and give an indication of the water quality located in wildlife areas such as waterfowl production areas. Documenting this distribution will ultimately improve our understanding of the fate and potential effects neonicotinoids may have on aquatic ecosystems in these regions, allowing natural resource managers, conservation groups as well as researchers the tools to develop strategies and policy to improve wetland water quality in agriculturally intensive regions such as the PPR.

3.3. Methods

3.3.1. Study Area

Our study was conducted within several counties located in the western portion of Minnesota's Prairie Pothole Region (PPR) (Figure 3.1). Sampled wetlands were located on Waterfowl Production Areas, managed by the U.S. Fish and Wildlife Service's Morris Wetland Management District. To limit variation in landform geomorphology and precipitation, our study focused on wetlands located within the North Central Glaciated Plains ecological region (ECOMAP 1993). The study locations were selected across a gradient of agricultural land use intensity.

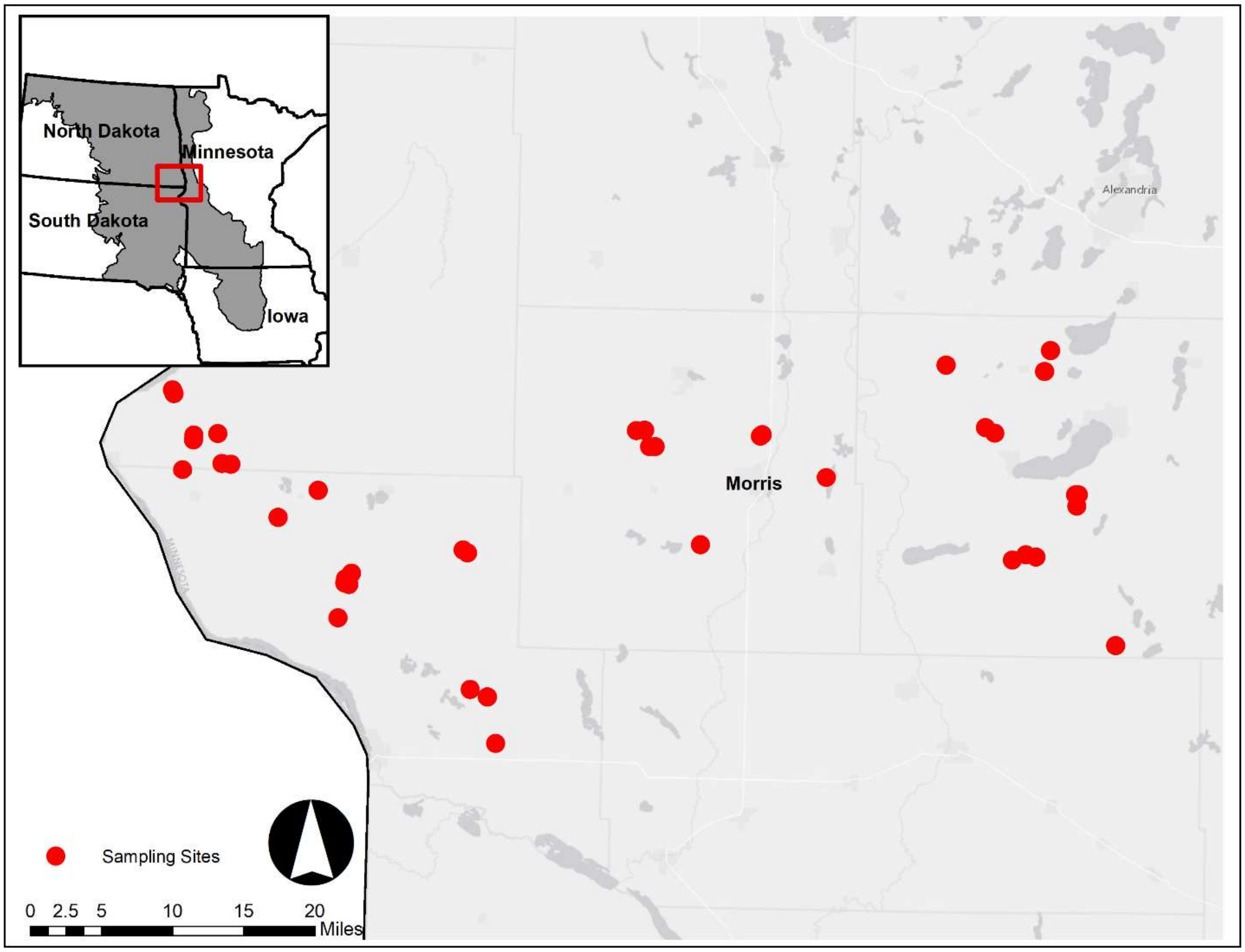


Figure 3.1. Location of sites in West Central Minnesota sampled for neonicotinoids in spring and early summer of 2017.

3.3.2. Wetland Selection

Study wetlands were selected based on their permanence and basin acreage following the classification system of (Cowardin et al. 1979) We identified all seasonal and semi-permanent basins ranging between 2-25 acres (.8-10 ha) in size. In this region, corn and soybean production are the dominant commercial crops, both of which utilize neonicotinoid pesticides, primarily as seed treatments (Douglas &Tooker 2015, Hladik et al. 2014) To estimate the basins susceptibility to potential neonicotinoid contamination we compiled data on crop cover from

2012-2015 from the USDA's Cropland data layer (USDA 2012-2015) and generated a 500 meter buffer around each basin utilizing GIS software (ArcGIS 10.4). Compiled land use data were calculated through the Geospatial Modeling Environment (GME) which uses the open source statistical software R and ArcGIS (Beyer 2015, R Core Team 2015). Output from the software provided estimates of the percent cropland to non-cropland which was used to classify basins according to crop intensity as a low crop (<25%), moderate crop (25-75%) and high crop (>75%).

In 2017, 40 randomly selected basins stratified based on land use and wetland permanence were sampled for neonicotinoids. Wetland water samples were collected from each basin on three separate occasions, ensuring sample timing was in accordance to the current agricultural activities taking place on the landscape. Since previous studies (Hladik et al. 2014, Main et al. 2014) have shown that neonicotinoid levels tend to be the highest during the early spring and summer months, our sampling effects were concentrated during these times. Our first sample took place in April, between snowmelt and planting activities to account for the potential runoff of neonicotinoids in snowmelt (Main et al. 2016a). Following updates from the Minnesota Crop Progress and Condition report (USDA 2017) we conducted our second sampling event near the end of May, when 94 percent of corn and 74 percent of the soybean crop had been successfully planted. Our final sampling efforts occurred during the early part of the growing season, in the month of June. Water samples were collected at each sampling location by submersing a 1 liter amber Nalgene bottle to a depth of 10 cm, beyond emergent vegetation where possible. In the field, samples were stored on ice and were frozen until analysis. General water quality measures including temperature, pH, conductivity, dissolved oxygen and turbidity were also collect during each sampling event using an YSI model 6920 sonde.

3.3.3. Chemical Analysis

Wetland water samples were analyzed for neonicotinoids at the Mississippi State Chemical Laboratory, Mississippi State University (Starkville, Mississippi) by liquid chromatography coupled with tandem mass spectrometry detection (LC/MS/MS). Quantifications were performed using external calibration standards using certified standard reference material. Samples were analyzed for five neonicotinoid compounds: imidacloprid, thiamethoxam, clothianidin, acetamiprid and thiacloprid.

3.4. Results

Following the survey of wetlands, water sample results indicated widespread distribution of neonicotinoids within the wetland management district. Overall 50% (20/40) of the wetland basins that were sampled for neonicotinoids tested positive for at least one compound throughout the multiple sampling events. Of the total 120 wetland water samples analyzed during the study a detectable level of at least one compound was identified in 29% (35/120) of the total water samples collected. Samples containing a mixture of multiple compounds were common with 34% (12/35) of the detectable samples containing at least two neonicotinoid compounds. The mean total neonicotinoid concentration of samples with detectable concentrations was 14.7 ng/L and the max concentration was 60 ng/L (Table 3.1). Three out of the five neonicotinoid compounds (clothianidin, thiamethoxam, imidacloprid) tested were detected in water samples throughout our sampling events during the spring and summer of 2017. Only clothianidin was present throughout the three separate sampling events while the others were detected in only two of the sampling periods (Table 3.2). Overall the three detected compound concentrations were relatively similar throughout the multiple sampling events (Figure 3.2). Clothianidin was the most detected neonicotinoid in water samples (24%) but had the lowest mean and max concentration (mean: 8.6 ng/L; max: 37) compared to the other detected compounds, imidacloprid (mean: 13.1 ng/L; max 38) and thiamethoxam (mean: 10.6 ng/L; max: 60).

Table 3.1**.** Summary of detection, arithmetic means and maximum concentrations of total neonicotinoids and active ingredients in water from prairie wetlands of West Central Minnesota. Total of 120 samples across three sampling events.

Compound	**Samples Detected**	**Detection Freq. (%)**	**mean (ng/L)**	**max (ng/L)**
Total Neonicotinoid (ng/L)[1]	35	29	14.7	60.0
Imidacloprid (ng/L)	8	7	13.1	38.0
Thiamethoxam (ng/L)	15	13	10.6	60.0
Clothianidin (ng/L)	29	24	8.6	37.0

[1]Total neonicotinoid concentrations are the sum of all three active ingredients detected in water samples

Table 3.2**.** Summary of detection, arithmetic means and maximum concentrations of total neonicotinoids and active ingredients throughout different sampling periods in water from prairie wetlands of West Central Minnesota. In addition to the three neonicotinoids listed, acetamiprid and thiacloprid were also measured, but no detections were reported from any sample period.

Sample Timing 2017			**Detection (%)**	**Total Neonic. (ng/L)[1]**		**Imidacloprid (ng/L)**		**Thiamethoxam (ng/L)**		**Clothianidin (ng/L)**	
Pre-Planting	**Crop Presence**	**Wetlands (n)**		**Mean**	**max**	**Mean**	**Max**	**Mean**	**Max**	**Mean**	**Max**
	Low	10	0	ND	ND	ND	ND	ND	ND	ND	ND
	Mid	20	5	2.0	2.0	ND	ND	ND	ND	2.0	2.0
	High	10	10	2.0	2.0	ND	ND	ND	ND	2.0	2.0
Planting > 80%	Low	10	10	2.0	2.0	2.0	2.0	ND	ND	ND	ND
	Mid	20	40	16.5	58.0	23.0	38.0	4.4	6.0	8.2	17.0
	High	10	70	15.9	60.0	ND	ND	24.0	60.0	6.5	12.0
Post-Planting	Low	10	10	2.0	2.0	ND	ND	ND	ND	2.0	2.0
	Mid	20	40	15.1	45.0	13.0	17.0	5.7	10.0	11.1	26.0
	High	10	80	17.8	41.0	4.0	4.0	12.0	20.0	10.8	37.0
Overall		120	29	8.1	60.0	4.7	38.0	5.1	60.0	4.7	37.0

[1]Total neonicotinoid concentrations are the sum of all three active ingredients detected in water samples

ND: not detected; reporting limit 2 ng/L for all active ingredients

Sample timing and the amount of cultivated crop near each basin played a role in the number of detections and the concentration levels found within a water sample (Table 3.2). Pre-planting detections were very low, with a detection frequency of only 5% (2/35). The detection frequency of neonicotinoids was highest during our post planting survey event (Figure 3.3a). Roughly 49% (17/35) of the positive detections occurred during this time, with the planting phase accounting for another 46% (16/35) of the positive detections. Total neonicotinoid concentrations also followed a similar pattern during the 2017 sampling season (Figure 3.3b). The concentration found during the pre-planting phase was low with a mean concentration of 2.0 ng/L. By contrast, neonicotinoid concentrations were highest during the planting phase and post planting with mean concentrations of 15.3 ng/L and 15.6 ng/L, respectively.

Land use within the buffer area also influenced the detection and concentration of the samples. Areas classified with a high amount of cropping intensity had the greater detection frequency with a 70% and 80% detection rate during the planting and post planting phases (Figure 3.3a). These areas also were found to have relatively high concentration levels with mean values of 15.9 and 17.8 ng/L, which were in relation to the planting and post planting survey events. Areas under moderate cropping intensity still had a number of detections both during the planting and post planting phases, with a detection frequency of 40%, throughout both time periods. Mean concentrations were similar to the levels found within the high intensity regions with mean concentrations of 16.5 and 15.1 ng/L. Low cropping activity sites, had very few detections and concentrations that were detected were low. Overall, based on our study results we noticed a positive correlation between land use and the total neonicotinoid concentrations $r_s = .39$, $p < .05$ found within our study wetlands (Figure 3.4).

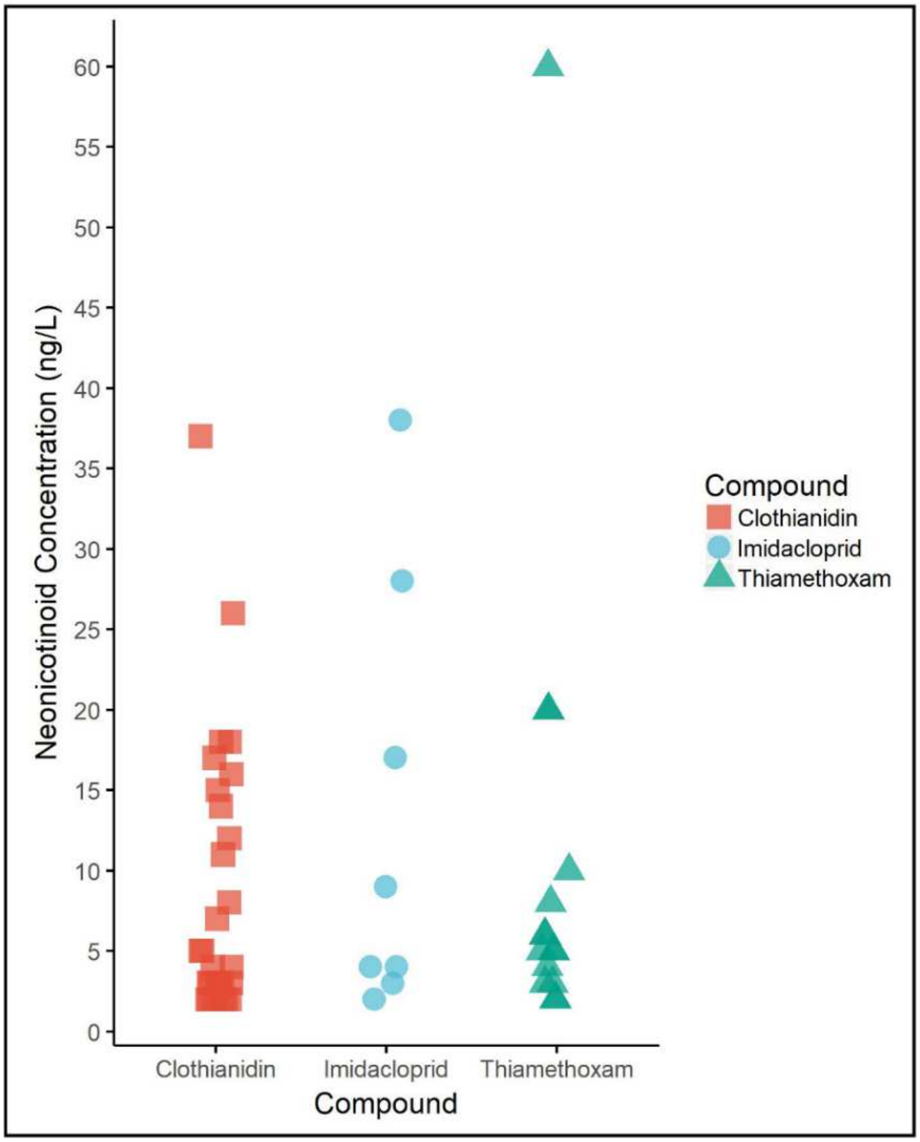
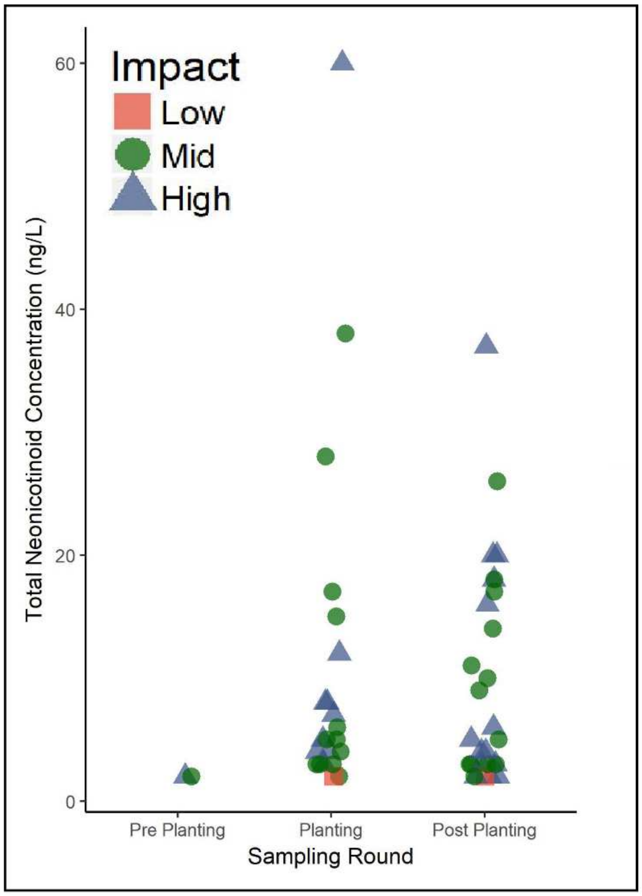
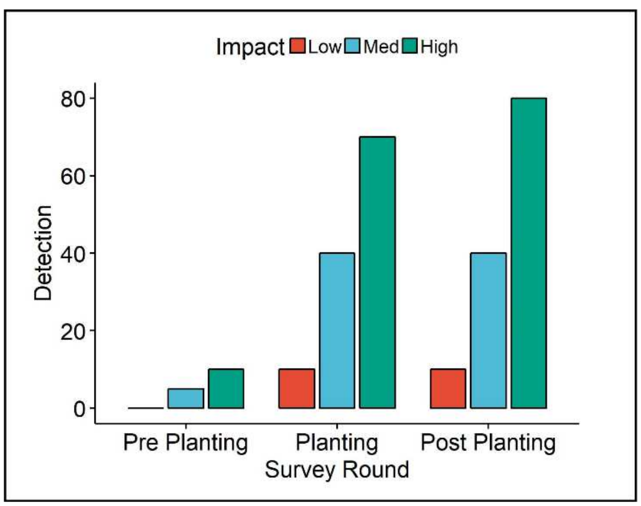


Figure 3.2. Distribution of neonicotinoid concentrations of each active ingredient detected following the survey of prairie wetlands in the spring and summer of 2017 (all three sampling periods).



b)



a)

Figure 3.3. Summary of (a) detection frequencies and (b) detection concentrations of total neonicotinoids in relation to planting activity and agricultural intensity collected during the spring and summer of 2017. Low (<25%), moderate (25-75%) and high (>75%) categories represent basin cropping intensity, based on crop cover data from 2012-2015.

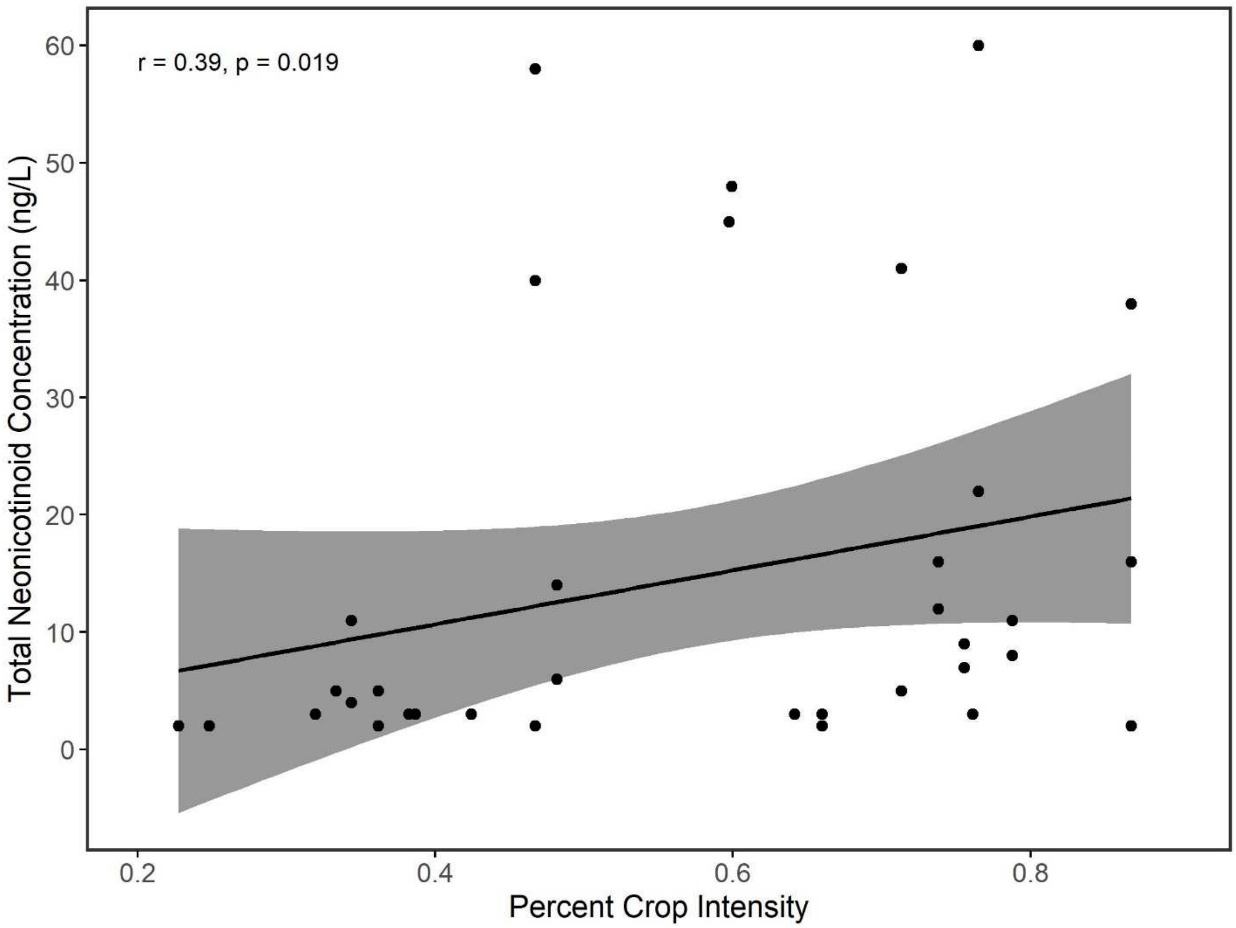


Figure 3.4**.** Relationship between the detected total neonicotinoid concentrations and the amount of cultivated crops within the 500-meter buffer area.

3.5. Discussion

To our knowledge, this is the first study that specifically assessed the distribution of neonicotinoid insecticides on federally managed waterfowl production areas in west central Minnesota. Detected levels of neonicotinoids in surface waters have been shown to be highly variable from region to region and previous studies have focused primarily on rivers, streams and drainage ditch systems (Hladik &Kolpin 2015, Starner &Goh 2012, Struger et al. 2017) with a series of studies focusing solely on wetlands in Canadas Prairie Pothole Region (Main et al. 2014, Main et al. 2016b, Main et al. 2015). The majority of the research sites studied by Main et al. (2014, 2015; 2016b) were located directly in agricultural fields as opposed to our study, where our research sites were located within Waterfowl Production Areas. Our results from sampling WPAs, indicates that wetlands found on protected habitats such as these are not immune to the off-site transport of neonicotinoids on the landscape. Overall 29% (35/120) of our water samples had detectable levels of neonicotinoid pesticides as well as half of the basins tested positive for a least one compound throughout the three sampling events.

Sites sampled during our study concentrated solely on basins surrounded by at least a portion of its upland habitat intact with grassland vegetation, resulting in observed concentrations lower than reported values in wetlands within the Prairie Pothole Region and surveys for neonicotinoids in other aquatic ecosystems across North America. Main et al. (2014) found concentrations of four neonicotinoid compounds in wetlands within cropped fields to have a mean concentration of 91.7 ng/L and a maximum concentration as high as 3110 ng/L, compared to an average and max total neonicotinoid concentration of 14.7 ng/L and 60 ng/L, respectively. Drained wetlands in the PPR of Iowa also showed detectable levels of neonicotinoids (Evelsizer &Skopec 2016) at levels exceeding both our study and the study conducted by Main et al. (2014). Since the wetlands studied by Evelsizer and Skopec (2016) were no longer functioning as intact wetlands and subject to traditional farming practices, it was not unexpected to find levels of concentrations an order of magnitude greater than values reported in this study. These results suggest that wetlands containing a significant portion of perennial cover surrounding the basin may be attributed to the lower concentrations found during our study. While our study area contains many Waterfowl Production Areas spread across the region, a large majority of the area consists of row crop agriculture dominated by corn, soybeans, wheat, and sugar beets. Row crop agriculture can have a major influence on the occurrence of active ingredients and based on the land use gradient used during our study we observed high detections and concentration of neonicotinoids in areas receiving moderate (25-75%) to high (<75%) cropping intensity within a specified distance to the wetland (Figure 3.4). Other studies of wetlands have found similar results, with an increased presence of contaminants between buffered and non-buffered wetlands (Osborne &Kovacic 1993, Riens et al. 2013).

As observed in other studies of neonicotinoid distribution across North America, the three most common active ingredients; imidacloprid, clothianidin and thiamethoxam were the compounds detected during our survey. Transport of neonicotinoids into wetlands via snowmelt has been shown to be a major driver of detectable concentrations of active compounds in wetland surface waters prior to spring planting activities (Main et al. 2016b). However, this was not particularity evident at our sites during the 2017 sampling season with only 2 of the 40 basins having a detectable concentration prior to planting. This may have been due to the early loss of snow from the landscape during the spring of 2017, while wetlands were still frozen, resulting in less transport of pesticides during spring thaw. As the ground was still frozen when the majority of snow melted, meltwater may not have percolated through soil and neonicotinoids present in the soil from surrounding agricultural activities may not have been readily transported into our wetlands during snowmelt. Our second and third samplings, which followed periods of precipitation, resulted in our highest observed detections and concentration of neonicotinoids. Precipitation events coinciding with planting activities during the spring and early summer has also been a common mechanism for the transport of neonicotinoids to nearby surface waters with previous studies observing a similar pattern (Hladik et al. 2014, Struger et al. 2017). Struger et al. (2017) found a positive correlation between active ingredients and the sampling day following rainfall events, highlighting the importance of sample timing in an effort to assess peak runoff events. Additional sampling should be focused in understanding how persistent these chemicals are, and to what extent they remain in wetlands over the growing season.

Agricultural drainage was evident at several of our survey sites, which could potentially help explain the transport of neonicotinoids onto some of the waterfowl production areas. While surface water run-off can be a major driver, sub-surface tile drainage can also contribute to the delivery of neonicotinoids to nearby wetlands, especially if they outlet directly into the basin or nearby drainage ditch. Neonicotinoid use throughout the region is primarily in the form of seed treatments and when subjected to seasonal rains in the spring and early summer, compounds have been shown to directly move into tile systems providing a preferential flow of neonicotinoid contaminated water to nearby sites (Chrétien et al. 2017, Wettstein et al. 2016). Small streams and agricultural ditch systems can also provide another exposure route of neonicotinoid contaminated water (Starner &Goh 2012, Struger et al. 2017). Many of these small systems, which can be primarily fed by agricultural runoff can travel throughout an area, interconnecting basins across the landscape. Often these streams and drains either empty or travel throughout these waterfowl production areas subjecting organisms to repeated pulses of multiple active ingredients which can display cumulative toxicities to organisms (Maloney et al. 2017). Throughout our survey we observed several of our study sites containing mixtures of neonicotinoids with 34% (12-35) of our detected samples having at least two compounds.

Neonicotinoids are thought to be linked to the declines of a variety of organisms, with much attention on bees and other native pollinator species (Hallmann et al. 2014, Hladik et al. 2016, Krupke et al. 2012). However, in wetlands and other surface waters experiencing contamination by neonicotinoids, non-target organisms such as aquatic insect species also have the potential to experience both acute and chronic concentrations, affecting both emergence success and sex ratios (Cavallaro et al. 2017). Waterfowl production areas which are managed primarily for breeding and migratory waterfowl, rely heavily on the availability of aquatic insects during times of breeding and migratory activities (Danell &Sjöberg 1977). Long-term exposure of neonicotinoid concentrations exceeding 35 ng/L have been shown to potentially impact sensitive aquatic invertebrate populations through chronic effects (Morrissey et al. 2015). While only 7 of our total 40 sites where found to contain concentrations above this critical value, it does provide evidence that areas considered protected are still impacted by the transport of neonicotinoids to nearby surface waters. Our research as well as others (Main et al. 2015) have shown that maintaining buffers of grassland habitat can be an effect way to reduce neonicotinoid concentrations in prairie pothole wetlands, but the design and effectiveness of buffer regions may vary.

3.6. Conclusion

Monitoring of prairie wetlands throughout western Minnesota indicated the presence of three of the most commonly used neonicotinoids in the region (USGS 2014). Out of the five active ingredients tested, clothianidin, imidacloprid and thiamethoxam were all found to occur in sampled wetlands during the 2017 sampling season. The results found in this study corroborate with other studies conducted throughout similar regions of North America and confirm the widespread distribution of these compounds within the environment. As expected, land-use intensity was positively correlated with detection concentrations of these pesticides at our study sites, with higher concentrations of neonicotinoids found in areas with a higher percentage of the surrounding watershed used for agriculture. In addition to the widespread occurrence of these compounds across our study region, comparison of concentration data from our three survey periods to published aquatic benchmark values indicate that wetlands found on waterfowl production areas can contain concentrations that exceed the suggested chronic toxicity benchmark set for imidacloprid. The current benchmarks set by the U.S. Environmental Protection Agency are 385 and 10 ng/L (USEPA 2018) for acute and chronic toxicity with similar thresholds of 200 and 8.3 ng/L, respectively, set by the European Water Framework Directive (Smit et al. 2015).

In addition to several of our wetlands exceeding chronic thresholds, a number of our sites also tested positive for multiple active ingredients. As stated previously, little is known about the potential toxicity of mixtures of neonicotinoids on aquatic organisms, with recent research (Maloney et al. 2017) indicating that simple additivity is no longer acceptable in term of toxicity. While our study did not test for other commonly used agrochemicals, research has shown that other chemicals such as fertilizers, herbicides and other insecticides can also be common in wetlands sounded by agricultural production (Evelsizer &Skopec 2016, Riens et al. 2013). Evaluating such effects to aquatic organisms can be a challenge to scientists, however, we suggest that further research continue to examine the fate of agrochemicals in prairie wetland ecosystems and develop an understanding of their impacts to aquatic ecosystem communities.

3.7. Acknowledgments

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APPENDIX. EMERGENCE COUNT DATA FROM 20 MESOCOSM TANKS SAMPLED DURING SUMMER OF 2016[1]

Tank	Species	Individuals Per Tank
1		
	Chaoborus	4
	Chironomidae	6
	Chironomini	92
	Chironomus	1
	Cladopelma	3
	Corynoneura	46
	Cricotopus	8
	Dicrotendipes	10
	Endochironomus	9
	Glyptotendipes	2
	Labrundinia	17
	Micropsectra	8
	Parachironomus	50
	Procladius	107
	Tanytarsini	472
	Tanytarsus	109
2		
	Ablabesmyia	3
	Chaoborus	2
	Chironomini	33
	Corynoneura	263
	Cricotopus	21
	Dicrotendipes	7
	Glyptotendipes	1
	Labrundinia	21
	Limnophyes	1
	Micropsectra	2
	Parachironomus	11
	Procladius	31
	Tanytarsini	48
	Tanytarsus	37
	Zavreliella	2
3		
	Chaoborus	12
	Chironomini	15
	Corynoneura	135
	Cricotopus	2
	Micropsectra	1
	Orthocladiini	1
	Parachironomus	10
	Polypedilum	1

Tank	Species	Taxon Per Tank
	Procladius	32
	Psectrotanypus	3
	Tanytarsini	18
	Tanytarsus	11
4		
	Ablabesmyia	2
	Chironomini	8
	Corynoneura	62
	Cricotopus	1
	Dicrotendipes	3
	Orthocladiini	1
	Parachironomus	2
	Procladius	3
	Tanytarsini	14
	Tanytarsus	10
5		
	Ablabesmyia	1
	Chironomidae	3
	Chironomini	31
	Cladopelma	2
	Corynoneura	55
	Cricotopus	5
	Cryptotendipes	1
	Dicrotendipes	8
	Labrundinia	2
	Limnophyes	1
	Orthocladiini	1
	Parachironomus	14
	Polypedilum	2
	Procladius	46
	Tanytarsini	57
	Tanytarsus	37
	Zavreliella	2
6		
	Chaoborus	9
	Chironomidae	1
	Chironomini	18
	Chironomus	1
	Corynoneura	141
	Cricotopus	7
	Dicrotendipes	3
	Endochironomus	1
	Labrundinia	5
	Lauterborniella	1
	Orthocladiini	1

Tank	Species	Individuals Per Tank
	Parachironomus	15
	Polypedilum	1
	Procladius	22
	Psectrocladius	1
	Tanytarsini	26
	Tanytarsus	20
7		
	Ablabesmyia	1
	Chironomidae	4
	Chironomini	34
	Cladopelma	1
	Corynoneura	86
	Cricotopus	11
	Dicrotendipes	1
	Labrundinia	4
	Parachironomus	27
	Polypedilum	3
	Procladius	36
	Tanytarsini	31
	Tanytarsus	9
8		
	Ablabesmyia	2
	Chaoborus	1
	Chironomidae	3
	Chironomini	52
	Cladopelma	1
	Corynoneura	159
	Cricotopus	6
	Dicrotendipes	18
	Labrundinia	3
	Micropsectra	1
	Parachironomus	31
	Procladius	10
	Pseudochironomus	1
	Tanytarsini	9
	Tanytarsus	5
9		
	Chaoborus	6
	Chironomidae	4
	Chironomini	37
	Cladopelma	8
	Corynoneura	11
	Cricotopus	1
	Cryptochironomus	5
	Dicrotendipes	19

Tank	Species	Individuals Per Tank
	Endochironomus	4
	Glyptotendipes	2
	Labrundinia	11
	Micropsectra	1
	Parachironomus	21
	Polypedilum	11
	Procladius	62
	Tanytarsini	100
	Tanytarsus	84
	Zavreliella	1
10		
	Ablabesmyia	1
	Chaoborus	2
	Chironomini	23
	Chironomus	1
	Cladopelma	3
	Corynoneura	46
	Cricotopus	9
	Cryptochironomus	3
	Dicrotendipes	3
	Glyptotendipes	2
	Parachironomus	8
	Procladius	35
	Tanytarsini	55
	Tanytarsus	39
	Zavreliella	11
11		
	Chaoborus	2
	Chironomidae	3
	Chironomini	46
	Corynoneura	66
	Cricotopus	16
	Cryptochironomus	2
	Dicrotendipes	1
	Labrundinia	10
	Parachironomus	37
	Procladius	15
	Tanytarsini	51
	Tanytarsus	23
12		
	Ablabesmyia	1
	Chaoborus	2
	Chironomidae	3
	Chironomini	41
	Chironomus	1

Tank	Species	Individuals Per Tank
	Corynoneura	82
	Cricotopus	5
	Dicrotendipes	4
	Endochironomus	1
	Parachironomus	16
	Procladius	2
	Tanytarsini	25
	Tanytarsus	11
13		
	Chaoborus	2
	Chironomidae	3
	Chironomini	26
	Chironomus	3
	Corynoneura	182
	Cricotopus	49
	Dicrotendipes	5
	Orthocladiini	1
	Parachironomus	19
	Procladius	2
	Tanytarsini	221
	Tanytarsus	20
14		
	Ablabesmyia	1
	Chaoborus	12
	Chironomidae	8
	Chironomini	48
	Chironomus	1
	Corynoneura	230
	Cricotopus	16
	Cryptochironomus	2
	Dicrotendipes	6
	Labrundinia	16
	Micropsectra	6
	Orthocladiini	5
	Parachironomus	20
	Polypedilum	7
	Procladius	17
	Psectrotanypus	2
	Tanytarsini	375
	Tanytarsus	177
	Zavreliella	1
15		
	Ablabesmyia	4
	Chaoborus	7
	Chironomidae	3

Tank	Species	Individuals Per Tank
	Chironomini	28
	Corynoneura	110
	Cricotopus	6
	Dicrotendipes	2
	Glyptotendipes	1
	Labrundinia	3
	Limnophyes	1
	Micropsectra	3
	Parachironomus	16
	Procladius	14
	Tanytarsini	60
	Tanytarsus	22
16		
	Chaoborus	11
	Chironomidae	3
	Chironomini	17
	Corynoneura	94
	Cricotopus	1
	Labrundinia	10
	Micropsectra	3
	Orthocladiini	1
	Parachironomus	16
	Procladius	27
	Tanytarsini	24
	Tanytarsus	26
17		
	Ablabesmyia	7
	Chaoborus	13
	Chironomidae	4
	Chironomini	36
	Chironomus	1
	Cladopelma	1
	Corynoneura	364
	Cricotopus	25
	Cryptochironomus	1
	Dicrotendipes	2
	Labrundinia	2
	Micropsectra	4
	Orthocladiini	2
	Parachironomus	17
	Paratendipes albimanus	2
	Polypedilum	5
	Procladius	54
	Tanytarsini	241
	Tanytarsus	79

Tank	Species	Individuals Per Tank
18		
	Chironomidae	10
	Chironomini	14
	Corynoneura	241
	Cricotopus	9
	Dicrotendipes	5
	Labrundinia	8
	Parachironomus	10
	Procladius	11
	Tanytarsini	144
	Tanytarsus	29
	Zavreliella	2
19		
	Ablabesmyia	1
	Chaoborus	8
	Chironomidae	2
	Chironomini	27
	Corynoneura	127
	Cricotopus	10
	Dicrotendipes	2
	Labrundinia	1
	Orthocladiini	1
	Parachironomus	22
	Procladius	12
	Tanytarsini	51
	Tanytarsus	7
20		
	Chironomini	24
	Corynoneura	95
	Cricotopus	1
	Orthocladiini	2
	Parachironomus	10
	Procladius	1
	Tanytarsini	19
	Tanytarsus	6

[1] Summary of number and species of chironomidae emerging from experimental mesocosm tanks between May 16, 2016 and August 9, 2016. Tanks 1, 5, 9, 13, and 17 are control tanks. 2, 6, 10, 14 and 18 received the low treatment dose (0.2µg/L) of imidacloprid. Tanks 3, 7, 11, 15 and 19 received the medium dose (2.0 µg/L) and tanks 4, 8, 12, 16, 20 the high treatment of 20.0 µg/L.