INSECTICIDES ALTER THE ABILITY OF PREDATOR ASSEMBLAGES TO SUPPRESS PREY

By

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April, 2019

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Agrochemicals are often deposited into freshwater bodies via rainfall and snowmelt events and can have consequences for populations and communities. Insecticides in aquatic systems can cause mortality, behavioral, and reproductive changes in nontarget invertebrates, which have the potential to indirectly effect other populations and ecosystem processes. Thiamethoxam (TMX) is part of the neonicotinoid family, a branch of insecticide that mimics nicotine and binds to the acetylcholine receptor, causing paralysis and death. This research examined 1) the effects of environmentally relevant concentrations of TMX on the diversity, species composition and total abundance of aquatic predatory insects present in freshwater ponds, and 2) how the effects of TMX on each of the diversity, species composition and total abundance of aquatic predatory insects may compromise the ability of the predator assemblage to suppress their prey. I found that, in the absence of TMX, the aquatic predator assemblage present is very effective at suppressing snail population growth. The ability of the predator assemblage to suppress prey, however, is severely reduced in the presence of TMX. TMX reduced the number of predatory insect species present, changed the species composition of the

predatory insects present, and reduced the total abundance of predatory insects present. Hemipteran insects appear to be more vulnerable to TMX than Odonates. It appears that a reduction in predator abundance had a minor effect in weakening the ability of the predator assemblage to suppress prey. Furthermore, the effect of species loss, in general, appeared to have little effect on the ability of the predator assemblage to suppress prey. Instead, it appears that the loss of one particular species of predator (*Belostoma*) as the result of TMX exposure is what mostly likely compromised the ability of the predator assemblage to suppress prey. Given the greater vulnerability of *Belostoma* to TMX, this means that TMX exposure will likely compromise the ability of the predator assemblage to suppress prey.

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A Thesis

Presented to the Faculty of the Department of Biology

East Carolina University

In Partial Fulfillment of Requirements for the Degree

Master of Science in Biology

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ACKNOWLEDGEMENTS

I would like to thank Dr. Chalcraft for his endless support and guidance throughout this process. Dr. Chalcraft has taught me valuable lessons in ecology and academics, from identifying macroinvertebrates to setting up cattle tanks to communicating results and ideas. I would like to thank the Chalcraft lab members, Dr. Suelen Calado-Tullio, Dr. Casey Nolan, and AJ Kitchen for their help throughout my time at ECU. I also thank my committee members, Dr. Heather Vance-Chalcraft and Dr. April Blakeslee, for feedback and guidance on my proposal and thesis. I especially would like to thank Dr. Vance-Chalcraft for helping me navigate PhD applications, and for her compassion and understanding. I would like to thank the many friends I have made during this process, and I am forever appreciative of the times we spent commiserating and working together to improve our science. I also thank ECU Biology Department for resources necessary to complete my experiments. Lastly, I would like to thank my husband, Matthew Schuster, for his unwavering support and kindness. Matt helped me ready my cattle tanks, collect invertebrates, and search through tanks at the end of the experiments in the heat of summer. Most importantly, Matt provided endless love and emotional support, and I could not have accomplished this goal without him. I am truly grateful for all the help I have received from my friends and family.

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CHAPTER I

INTRODUCTION

The widespread application of nitrates, phosphates, and pesticides on farms can result in water runoff that enters and pollutes bodies of water (Conley et al. 2009). Excess nitrates and phosphates can cause eutrophication and dead zones (Conley et al. 2009). Pesticides and insecticides contaminate soils and waterways in the US (Pisa et al. 2014). Various types of pesticides, including herbicides and fungicides, have been directly linked to biodiversity loss, and can travel through soils, ground water, and rainwater (Vogel et al. 2008), with groundwater and sediment-bound herbicides and pesticides being important drivers of ecotoxicity (McKnight et al. 2015). Input of these agrochemicals can have important effects on aquatic ecosystems. For example, cypermethrin (a pyrethroid insecticide) causes direct mortality to *Daphnia* and copepods (Friberg-Jensen et al. 2003), and imidacloprid (a neonicotinoid insecticide) has been shown to alter the behavior of *Gammarus pulex* by inhibiting feeding responses (Nyman et al. 2013). Mixtures of both imidacloprid and thiamethoxam caused a 10% decrease in successful emergence of *Chironomus dilutes* (Maloney et al. 2018). Contamination-induced changes in the survival, reproduction, or behavior on some species can also have indirect effects on other species in the community, including a release from competitive interactions or predation (Fleeger et al. 2003). Indirect changes include both top-down (predator-influence on lower levels) and bottom-up (prey-influence on higher levels) trophic cascades (Pace et al. 1999). For example, Atrzine and Endosulfan can indirectly alter the abundances of some species in aquatic food webs

by reducing the abundance of their food resources or the abundances of competing species that die when exposed to the pesticides (Rohr and Crumrine 2005).

Some pesticides have been developed to target insects so as to not harm vertebrates. Neonicotinoids are a fairly new class of insecticide that causes constant nervous system stimulation by binding to and activating the post-synaptic nicotinergic acetylcholine receptors (nAChR). Vertebrates and invertebrates differ in the structure of nAChR present in them which causes the neonicotinoid bond to be weak and temporary in vertebrates but not in invertebrates (Yamamoto et al. 1995). Consequently, neonicotinoids have been praised for having relatively little impact on non-target vertebrates (Tomizawa and Casida 2003).

Since its discovery in the 1990's neonicotinoids have become major components of the pesticide market. The neonicotinoid family made up 17% of the insecticide market worldwide and accrued $1.56 billion in sales (Jeschke and Nauen 2008) in 2006, and by 2010 neonicotinoids had jumped to 26% of the global market (Simon-Delso et al. 2015). The most common neonicotinoid, and one of the first discovered, is imidacloprid. Thiamethoxam (TMX) and dinotefuran are more recently developed neonicotinoids that are used increasingly across the United States (Pesticide National Synthesis Project 2015). In 2015 TMX was used in all 48 continental states, and it is used most commonly for corn and soybeans, crops that are frequently grown on North Carolina farms (Pesticide National Synthesis Project 2015). There are approximately 2.5 million all-purpose acres of soybean and corn in North Carolina (Census of Agriculture). TMX can be applied through spraying, seed application, or through irrigation systems (only for potatoes) but can drift while spraying, leach into ground water via porous soils, and/or runoff into water systems (Actara product label).

A recent review of studies conducted in 9 different countries revealed that neonicotinoids were present (geometric average and peak concentration of 0.13 μg/L and 0.63 μg/L; respectively) in most surface waters that received runoff from agricultural areas (Morrissey et al. 2015). Although surface water contamination data for TMX in North Carolina is not available, playa lakes in Texas have been found to have an average and peak TMX concentration of 3.6 μg/L and 225 μg/L; respectively (Anderson et al. 2013). Neonicotinoids most commonly reach surface waters from runoff after rainfall or snow melt and, unsurprisingly, their concentration in surface waters dramatically increase after rainfall events (Morrissey et al. 2015). Wetlands and rivers that receive runoff from agriculture have higher concentrations of neonicotinoids compared to rivers, streams, and lakes that do not receive runoff (Morrissey et al. 2015). The year-round presence of neonicotinoids in wetlands and rivers found in agricultural areas could be due to either a long persistence time in water bodies and soils and/or continuous application of the insecticide to agricultural areas (Morrissey et al. 2015). TMX has been shown to persist in soil for 7 to 72 days and has a half-life of 2.7-39.5 days in water when exposed to sunlight (MacBean 2010). Persistence in water can be lengthened with high turbidity and pH (Sarkar et al. 2001). Multiple kinds of neonicotinoids were often found in a single water sample in levels that exceeded suggested concentrations (Morrissey et al. 2015), supporting a need to understand effects from every type of neonicotinoid, as well as their combined effects on ecosystems.

It is generally accepted that neonicotinoids have lower vertebrate toxicity than invertebrates likely due to the shape of the acetylcholine receptors (Simon-Delso et al. 2015, Yamamoto et al. 1995), however, a few studies have demonstrated potential sublethal effects of exposure. Wood frogs exposed to imidacloprid as tadpoles were less likely to jump away from an attack as adults than individuals that were raised in the absence of imidacloprid, indicating that

high levels of imidacloprid could increase the risk of predation when the animals are no longer directly exposed to imidacloprid (Robinson et al. 2017). Imidacloprid has also been shown to disrupt neuronal activity in zebrafish, which can have a negative effect on brain activity (Özdemir et al. 2018). Dinotefuran (a neonicotinoid) and TMX have the potential to increase acetylcholine in Chinese lizards, eventually increasing a release of dopamine in the brain, however the insecticides were rapidly excreted, causing very short-term effects (Wang et al. 2019). The body of research examining potential sublethal effects of neonicotinoids of vertebrates still needs further development.

Although there is there is little evidence to suggest that environmentally relevant concentrations of TMX causes direct mortality in vertebrates, there is increasing evidence that TMX has toxic effects on non-target invertebrate species which can indirectly influence the population size of vertebrate species. Elevated levels of the toxic residues of imidacloprid are negatively correlated with the population size of species in a diverse array of invertebrate orders (e.g., Amphipoda, Actinedida, Basommatophora, Diptera, Ephemeroptera, Isopoda, Neotaenioglossa, Odonata, and Trichoptera)(Van Dijk et al. 2013). These reductions can also have implications for aquatic and terrestrial vertebrates. For example, farmland areas that have a lower abundance of aquatic invertebrate populations have also been found to have a lower abundance of insectivorous birds (Hallman et al. 2014).

Neonicotinoid pesticides have also been linked to sublethal impacts on nontarget invertebrates (Kessler et al. 2015). For example, neonicotinoid pesticides can increase the mortality risk and can cause motor impairment in bees (Kessler et al. 2015). Foraging bees are often exposed to both neonicotinoids and pyrethroid, and imidacloprid has been shown to reduce total worker production and brood production, and increase the probability of colony failure in

bumblebees (Gill et al. 2012). Other documented sublethal effects of neonicotinoids include feeding inhibition in mayflies (Alexander et al. 2007), a decrease in body growth of mosquitos (Ashauer et al. 2011), and immobility in mayflies and caddisflies when exposed to 0.1 to 0.2 μg/L of imidacloprid (Roessink et al. 2013). Nonlethal levels of clothianidin, the breakdown product of thiamethoxam, caused a 62% reduction in the rate of prey consumption by water bugs (Miles et al. 2017). Consequently, neonicotinoids appear to have the potential to greatly alter the efficiency in which predator insects capture their prey.

The susceptibility of individuals to TMX can vary among species (Miles et al. 2017); likely due to variation in the molecular structure of the nAChR (Yamamoto et al. 1995). For example, a meta-analysis revealed that Odonata are more susceptible (average LC50 of 55.2 μg/L) to neonicotinoids than are Hemiptera (average LC_{50} of 64.9 μg/L) or Decapoda (average LC50 of 1562.2 μg/L) (Morrissey et al. 2015). A study on 8 species of predatory invertebrates suggests that the order of sensitivity to clothianidin based on LC50 tests is 1) Coleoptera (based on *Graphoderus fascicollis*; LC50=0.002mg/L), 2) Hemiptera (based on *Hesperocorixa atopodonta;* LC50=0.056mg/L, *Notonecta undulata;* LC50=0.059mg/L, and *Belostoma flumineum;* LC50=0.079mg/L), 3) Decapoda (based on *Orconectes propinquus;* LC50=0.805mg/L), and 4) Odonata (based on *Plathemis lydia;* LC50=0.865mg/L, *Anax junius;* LC50=1mg/L, and *Lestes unguiculatus;* LC50=1.245mg/L) (Miles et al. 2017). Snails are relatively tolerant of neonicotinoids, with 0 mortality in *Physa* and *Heliosoma* when exposed to 327 mg/L clothianidin (Miles et al. 2017). A review of the LC_{50} literature reports that across all neonicotinoids Odonates have a geometric LC_{50} value of 0.0552mg/L, and Hemipterans have geometric LC_{50} value of 0.0649mg/L (Morrissey et al. 2015).

Given that TMX reduces the abundance of aquatic predatory insects and that different

aquatic predatory insect species may differ in their susceptibility to TMX, it is critical to evaluate how TMX induced changes to the collection of aquatic predatory invertebrates alters the ability of the predator assemblage to suppress the population size of their prey. There is generally a positive relationship between predator species richness and prey suppression, suggesting that more predator species in a community will lead to greater prey suppression (Griffin et al. 2013), except in cases of intra-guild predation (Vance-Chalcraft et al. 2007). A predator assemblage is often only as successful at suppressing prey as the strongest predator species in the assemblage (Griffin et al. 2013). Prey can also become more vulnerable to predation in the presence of multiple predators. When prey change their behavior or morphology to avoid predation by one predator, they can become more vulnerable to a different predator and enhance their overall mortality risk (Polis et al. 1989, Soluk 1993, Vance-Chalcraft and Soluk 2005, Swisher et al. 2006, Vance-Chalcraft et al. 2007). In some instances more diverse predator assemblages have a weaker effect on prey suppression. This can result as a consequence of intra-guild predation, in which predators consume other predator species, or as a result of interference competition, in which predators aggressively compete for a shared prey resource (Polis et al. 1989; Vance-Chalcraft et al. 2007). Generally, as the number of trophic linkages between predators increases, the ability of the predator assemblage to suppress prey decreases, and if intermediate predators are effective at suppressing prey the addition of a top predator typically results in prey release (Vance-Chalcraft et al. 2007). Predators that may be categorized in similar trophic levels or functional groups may not be identical in their capacity to suppress prey, or their preference for certain prey species (Kurzava and Morin 1998; Chalcraft and Resetarits 2003).

TMX has the potential to reduce the number of predator species present, the species composition of predators present, and the total number of predators present in aquatic

ecosystems, as demonstrated by LC_{50} concentrations. I hypothesized that TMX will alter the assemblage of predatory insects present in freshwater ponds by reducing the species richness and total abundance of aquatic predatory insects present. I also hypothesized that some predatory insect species will be more vulnerable to TMX and therefore alter the species composition of the insect species present. Given these expected changes in the predator assemblage, and the potentially important role that the total abundance of predators present, the species richness of predators present, and the identities of the predators present have on prey suppression, I expected that TMX will indirectly alter the ability of the predator assemblage to suppress their snail prey (Figure 1).

Two experiments were performed to test these hypotheses. The first experiment assessed how different concentrations of TMX affects the diversity, abundance and species composition of aquatic predatory invertebrates that are present in a pond food web. The second experiment assessed how TMX induced changes in the abundance, diversity and species composition of aquatic predatory invertebrates present affects the ability of the predator assemblage to suppress prey.

CHAPTER II

MATERIALS AND METHODS

Experiment 1

I conducted an experiment in 30 artificial ponds or mesocosms filled with 1000 l of water to assess how TMX affects the species richness, composition and abundance of aquatic predatory insects present and to assess the direct effects of TMX on their snail prey. The experiment implemented six treatments. Four treatments contained 6 individuals of each of four species of local aquatic macroinvertebrate predators (*Erythemis spp, Pachydiplax spp, Ranatra spp,* and *Belostoma spp,*) and 50 of their snail prey (*Physa spp*) but varied in the concentration of TMX present (0mg/L, 0.0077mg/L, 0.0634mg/L, or 0.225mg/L) and two treatments lacked predators but contained 50 snails and varied in the concentration of TMX present (0mg/L or 0.225mg/L). The treatments with predators allowed me to assess the effects of TMX on the species richness, composition and abundance of aquatic predatory insects present while treatments lacking predators were necessary to assess the direct effects of TMX on snails.

Environmentally relevant concentrations of TMX were determined using water samples from Canada and Texas, as there have not been published data on concentrations in North Carolina water bodies. The maximum concentration of TMX in samples from Texas playa lakes was 0.225mg/L (Anderson et al. 2013) and the maximum concentration of TMX found in Canadian puddles was 0.0634mg/L (Samson-Robert et al. 2014), which was used as the intermediate level. The average concentration of TMX found in Canadian water bodies was 0.0077mg/L (Samson-Robert et al. 2014), so that concentration was used for the lowest

concentration. Aquatic insect species used in the experiment are all native to Eastern North Carolina and are commonly found together in freshwater ponds without fish.

I created 30 artificial ponds out of 1100-liter cattle tanks and arranged them in five spatial groups (blocks) of six tanks each. All procedures were completed on mesocosms in a block by block fashion. After filling each mesocosm with 1000 L of well water, mesh lids were placed over the tanks to prevent unwanted colonization and emigration during the experiment. 500 g of pine leaf litter were added to each cattle tank, along with two ceramic tiles on the east and west sides of the bottom of the tank, leaning up on the sides at a 45-degree angle to provide additional refuge for prey. The tanks were then inoculated with 473ml of pond water containing zooplankton and phytoplankton from several ponds without fish around Greenville. I randomly assigned one of the six treatments to one tank within each of the five blocks so that each treatment was replicated five times. Beginning three days after plankton was added to all mesocosms, I implemented procedures to produce environmental conditions within each mesocosm that corresponded to the identity of the treatment that was randomly assigned to each mesocosm.

To manipulate the amount of TMX present in mesocosms, I placed an appropriate amount of Flagship 25WG into mesocosms assigned to a treatment that requires $\geq$ 0.0077mg/L of TMX present. Flagship 25WG comes in the form of granules in which 25% of the weight is TMX (the active ingredient of Flagship 25WG) so I multiplied the concentration of TMX required for a particular treatment by 4, and then by 1000 to determine the mg of Flagship 25WG to add to the tanks. Mesocosms were stirred after the addition of Flagship 25WG to ensure even distribution. Within 24 hours of initiating the TMX manipulations, I added 50 snails to each mesocosm and placed six individuals of each predator species into mesocosms that were

assigned treatments that required predators to be present. Predators and snails were collected from several freshwater ponds around both Greenville and the Croatan National Park near New Bern, North Carolina. The predators were collected over four days one week before the beginning of the experiment, and snails were collected two days before the experiment began.

Temperature extremes were measured daily, and cattle tanks were checked daily for dead individuals, metamorphosed individuals, leaks, and to make sure lids were still secure. Dead insects were preserved in ethanol. At the end of 15-19 days, a different day for each block, I drained the tanks and searched through collected leaf litter and inspected mesocosm walls for snails and predators. Predators and snails that remained alive were preserved in ethanol for each tank. I counted the total number of predators and predator species present in each mesocosm at the end of the experiment. I then compared those values to the number of individuals and predator species initially added to the mesocosms at the beginning of the experiment to quantify how many individuals and species were lost during the experiment.

I performed two linear mixed models to evaluate how TMX dose affects the number of predator species and the total number of predators that did not survive during the experiment. The independent variables for both models included treatment as a fixed effect and block as a random effect. Treatments without predators were not included in these analyses. I used planned comparisons (orthogonal polynomials) to assess trends in the relationship between dose and reductions in predator abundance (number of individuals) and diversity (number of species).

I also performed four linear mixed models (one for each predator species) to evaluate how TMX dose affects the number of individuals of each predator species that died during the experiment. These models included treatment (treatments containing predators) as a fixed effect and block as a random effect. Population growth rate (r) of snails in a particular mesocosm was

estimated as $\log(N/N_0)$ where N is the number of snails found at the end of the experiment and N_0 is the number of snails placed in a mesocosm at the start of the experiment. A linear mixed model was performed to analyze how TMX dose and the presence or absence of predators affected snail population growth rate using treatment as the fixed effect and block as a random effect. Planned comparisons were used to test the following: 1) snail per capita growth rate in the absence of predators, 2) linear contrast to assess linear effect of TMX on the ability of predators to suppress snails, and 3) quadratic contrast to assess nonlinear effect of TMX on the ability of predators to suppress snail per capita growth rate.

Experiment 2

In experiment, 2 I assessed how the degree to which TMX affected predator abundance, species richness and species composition in experiment 1 affected the ability of a predator assemblage to suppress prey (snails). In this experiment, I did not manipulate TMX but rather I focused on manipulating the predator assemblage in ways that corresponded to the influence that TMX had on the predator assemblage in experiment 1. Specifically, I found in experiment 1 that exposure to 0.0634mg/L of TMX reduced species richness by 2 species, total predator abundance by 6 individuals, and had a greater adverse effect on the survival of *Belostoma* and *Ranatra* than *Erythemis* and *Pachydiplax.* Consequently, experiment 2 examines the potential for TMX to indirectly alter snails via different ways in which TMX affects the predator assemblage.

This experiment was conducted in artificial ponds and included 10 treatments. All treatments required the presence of 50 snails in a pond. Two reference treatments include 1) a treatment with prey but no predators and 2) a treatment with prey and a collection of predators that co-occur with each other in the absence of TMX. The collection of predators (10 predators

present representing 4 predator species) that co-occur with each other in the absence of TMX was represented by the relative abundances of each predator species (2 *Belostoma* individuals, 2 *Ranatra* individuals, 3 *Pachydiplax* individuals, and 3 *Erythemis*) that survived to the end of the experiment in the treatment lacking TMX in experiment 1.

The remaining eight treatments corresponded to different ways in which TMX exposure could alter the predator assemblage. To assess the total indirect effect that TMX-induced changes in predator assemblages have on snails, I created a third treatment that corresponded to the predator assemblage found when an intermediate dose (0.0634mg/L) of TMX is present. Specifically, this treatment was represented by 4 predator individuals present (6 less than would occur with no TMX) representing 2 predator species (2 less than would occur with no TMX) that have better survival odds when exposed to TMX (2 *Pachydiplax* individuals and 2 *Erythemis* individuals). To assess how the extent to which TMX reduced the total abundance of predators affected snails, independently of changes in predator species richness, I established a fourth treatment (consisting of 1 *Belostoma*, 1 *Ranatra*, 1 *Pachydiplax*, and 1 *Erythemis*) that maintained the composition and richness of the community that would otherwise occur with no TMX present but reduced the total abundance of predators by 6 (i.e., the amount to which 0.0634mg/L of TMX reduced predator abundance).

To measure the effect that TMX-induced reductions in species richness had on a predator assemblage's ability to suppress prey, independent of changes in the total abundance of predators present, I created six other treatments that simulated the loss of two predator species (i.e., the amount of loss that occurs with exposure to 0.0634mg/L of TMX) but maintained the total number of predator individuals that would be found when no TMX exposure occurred. These six treatments differed in which two predator species remained and all of these six treatments

corresponded to all of the potential pairs of species that could derive from the initial assemblage that contained 4 predator species. The abundance of each species present in these two species treatments was set at five in order to maintain total predator abundance. This would equate to the idea that species remaining following TMX exposure can increase in abundance following the loss of other competing species. One of these six treatments with two species present was represented by the two species (*Pachydiplax* and *Erythemis)* that had a higher probability of surviving exposure to 0.0634mg/L of TMX. The other five treatments with two species present help to provide insight into whether it is the loss of particularly vulnerable species that compromises the ability of a predator assemblage to suppress prey or whether the ability of the predator assemblage to suppress prey is compromised by the loss of any two species. These 10 treatments were replicated 5 times for a total of 50 1100 cattle tanks.

Cattle tanks were arranged in five spatial blocks of tanks each, filled with 1000 L of well water, and mesh lids were kept on the tanks to prevent unwanted colonization and emigration. 300 g of pine leaf litter were added to each cattle tank, along with two ceramic tiles on the east and west sides of the bottom of the tank, leaning up on the sides at a 45-degree angle to provide additional refuge for prey. The tanks were then inoculated with 473ml of pond water containing zooplankton and phytoplankton from several ponds without fish around Greenville. Inoculation occurred sequentially for each block over 5 days. The tanks were then left for 3 days each to allow the plankton to establish. After the mesocosms were established I randomly assigned one of the 10 treatments to each mesocosm within a block such that all treatments were represented once within the block. Predators and snails were added to mesocosms in the same block on the same day but predators and snails were added to mesocosms in different, but sequential, days to allow each mesocosm to run for the same duration (12 days) and to provide me with a complete

day to sample each mesocosm in a block. After animals were present in mesocosms for 12 days, I emptied water from the mesocosm and searched the mesocosm walls and the litter to collect and preserve any remaining snails and predators in ethanol. Prey abundance and survival was measured by counting the number of snails in the tank. Snail reproduction was measured by counting the number egg masses laid on the sides of the tanks.

I analyzed snail survival and the number of snail egg masses present with generalized linear mixed models that included treatment as a fixed effect and block as a random effect. For the snail survival model, I used a logit link function but used a quasi-binomial approach to estimating variance because overdispersion resulted when assuming a binomial distribution. For the number of snail egg masses model, I used a log link function and employed a quasi-Poisson approach to estimate variance as assuming a Poisson distribution resulted in overdispersion. For both generalized linear mixed models, planned contrasts (Table 1) were used to test several hypotheses including 1) predators are effective in suppressing snails when not exposed to TMX, 2) the overall effect of TMX induced changes in the predator assemblage compromises the ability of the predator assemblage to suppress prey, 3) TMX-alters the ability of the predator assemblage to suppress prey compared to the ability of an unaltered predator assemblage, 4) a predator assemblage with few predators is able to suppress prey, 5) TMX reductions in total predator abundance compromises the ability of the predator assemblage to suppress snails, 6) reductions in predator species richness alone compromises the ability of the predator assemblage to suppress prey regardless of which predator species are lost, 7) reductions in predator species richness alone compromises the ability of the predator assemblage to suppress prey when the predator species most vulnerable to TMX are lost, 8a-f) each of the altered predator assemblages

are capable of suppressing prey, 9a-e) each of the altered predator assemblages have a different effect on prey than the unaltered assemblage.

CHAPTER III

RESULTS

Experiment 1

The number of species lost during the experiment differed among treatments that varied in TMX concentration ($F_{3,12}$=20.37, p<0.001) (Figure 2. No species were lost when the predator assemblage was exposed to no TMX but trend analysis revealed that species loss increased non-linearly with TMX dose (linear component: $F_{1,12}$=51.89, p<0.001; quadratic component: $F_{1,12}$=4.43, p=0.057; cubic component: $F_{1,12}$=4.78, p=0.049). Though species loss was greatest in treatments with the highest dosage of TMX, the non-linear response of species loss to TMX revealed that the species loss increased more rapidly with TMX dose across the lower concentrations of TMX than across the higher concentrations. At the intermediate dose (0.0634mg/L), TMX caused species richness to decline by 2 species.

The number of individuals lost during the experiment differed among treatments that varied in TMX concentration ($F_{3,12}$=32.71, p<0.001) (Figure 3). Trend analyses reveal that the number of predators lost increased non-linearly with TMX concentration (linear component: $F_{1,12}$=81.83, p<0.001; quadratic component: $F_{1,12}$=16.83, p=0.002; cubic component: $F_{1,12}$=0.0007, p=0.982). Specifically, there was only a very small increase in the number of predators lost as dosage initially increased, but the number of predators lost increased more substantially with higher dosages of TMX. At the intermediate dose of TMX, mesocosms lost approximately 5.6 more predators than treatments that did not contain TMX which corresponded to approximately 4 predators remaining after exposure to an intermediate amount of TMX.

The probability of an individual surviving in mesocosms clearly varied with TMX dose for all species of aquatic predatory insect except for *Erythemis*, where the relationship between survival and TMX dose was less clear (*Belostoma*: $F_{3,12}$=10.78, p=0.001, *Ranatra*: $F_{3,12}$=14.16 , p<0.001, *Pachydiplax*: $F_{3,12}$=6.78, p=0.006, *Erythemis*: $F_{3,12}$=2.34 p=0.125). A small increase in TMX dose caused a greater reduction in survival in *Ranatra* and *Belostoma* than it did in either *Erythemis* or *Pachydiplax* which suggests they are more vulnerable to TMX (Table 2). All species had very low survival at the highest dosage of TMX (Table 2).

The population growth rate of snails differed among treatments ($F_{5,20}$=3.65, p=0.017) (Figure 4). There is little evidence to suggest that TMX altered snail per capita growth rate in the absence of predators (t_{29}=-1.43 p=0.168) but there is reasonable evidence to suggest that predators suppress snail per capita growth rate in the absence of TMX by 38.57% (t_{20}=-1.97, p=0.063) (Figure 4). Among treatments with predators there was a nonlinear relationship between per capita growth rate and dose (linear component: $F_{1,12}$=3.66, p=0.08; quadratic component: $F_{1,12}$=0.41, p=0.532; cubic component: $F_{1,12}$=9.17, p=0.011). Specifically, snail per capita growth rate in the presence of predators changed from negative (indicative of a decline in population in size) when no TMX was present to a relatively consistent positive value when TMX was present.

Experiment 2

Snail survival

Variation in the predator assemblage present caused a change in prey survival ($F_{9,36}$=10.42, p<0.001). In the absence of predators, snail survival was high (76.35% +/- 8.51%)

(Figure 5). The predator assemblage that occurs when no TMX was present was effective in suppressing prey, reducing the percentage of snails surviving by 34.55% (t_{40}=-2.38, p=0.022) relative to the treatment with no predators (Figure 5). The TMX altered predator assemblage (in terms of richness, abundance, and composition) was not very effective at suppressing snail survival (t_{40}=0.01, p=0.989). Consequently, snail survival in the altered predator assemblage (4 individuals of 2 species) was substantially higher than that observed when the unaltered predator assemblage (10 individuals of 4 species) was present (t_{40}=-2.39, p=0.017).

Reducing the abundance of predators, independent of changes in species richness, increase snail survival by 23.46% in comparison to the unaltered predator assemblage but the evidence that this difference was statistically different from zero was weak (t_{40}=1.52, p=0.136) (Figure 5). Nonetheless, the probability of snails surviving when predator abundance was reduced and no change in species richness occurred was also not different from the probability of snails surviving when no predators were present (t_{40}=-0.86, p=0.397) even though there was stronger evidence that the unaltered predator assemblage suppressed prey survival (reported in the prior paragraph).

On average, reducing the number of predator species present from 4 to 2, while keeping total predator abundance constant at 10, did not result in snail survival (*Pachydiplax* and *Erythemis*: 56.13% +/- 11.61%; *Pachydiplax* and *Belostoma*: 6.29% +/- 2.78%; *Pachydiplax* and *Ranatra*: 73.06% +/- 9.28%; *Erythemis* and *Belostoma*: 18.09% +/- 6.99%; *Erythemis* and *Ranatra*: 80.34% +/- 7.44%; *Belostoma* and *Ranatra*: 15.36% +/- 6.13%) (t_{40}=0.44, p=0.661) to be substantially different from that observed in the unaltered predator assemblage (Figure 5). Instead, the effect of losing two predator species while maintaining predator abundance depended on which predator species were lost. The loss of the most vulnerable predator species

did not appear to compromise the ability of the predator assemblage to suppress snail survival when total predator abundance did not change (t_{40}=-0.91, p=0.367). In other scenarios, particularly those in which *Belostoma* was not one of the lost species, the loss of predator species richness while maintaining total predator abundance actually enhanced the ability of the predator assemblage to suppress snail survival relative to the unaltered predator assemblage (*Pachydiplax* and *Belostoma* (t_{40}=3.75, p<0.001); *Erythemis* and *Belostoma* (t_{40}=1.86, p=0.07); *Belostoma* and *Ranatra* (t_{40}=2.18, p=0.036)) and to the assemblage with no predators (*Pachydiplax* and *Belostoma* (t_{40}=-6.12, p<0.001); *Erythemis* and *Belostoma* (t_{40}=-4.24, p<0.001); *Belostoma* and *Ranatra* (t_{40}=-4.55, p=0<0.001)) Yet again, in other scenarios where at least one of the lost predator species was *Belostoma*, the loss of predator species while maintaining total predator abundance compromised the ability of the predator assemblage to suppress snail survival as snail survival was higher in those treatments than when the unaltered predator assemblage was present (*Pachydiplax* and *Ranatra* (t_{40}=-2.1, p=0.042); *Erythemis* and *Ranatra* (t_{40}=-2.75, p=0.009)). In fact, when species loss was associated with the loss of *Belostoma*, the ability of the predator assemblage to suppress snail survival was very weak as snail survival was not very different from that observed in the absence of predators (*Pachydiplax* and *Erythemis* (t_{40}=-1.46, p=0.151); *Pachydiplax* and *Ranatra* (t_{40}=-0.28, p=0.784); *Erythemis* and *Ranatra* (t_{40}=0.37, p=0.711)).

Number of snail egg cases

Patterns of differences among treatments in the abundance of snail casings were similar to the patterns reported for snail survival. Variation in the predator assemblage present caused a change in prey survival ($F_{9,40}$=4.91, p<0.001). In the absence of predators, snails produced 46.03 +/- 20.45 eggs masses (Figure 6). The predator assemblage that occurs when no TMX was

present was effective in suppressing snail reproduction, reducing the average number of egg masses by 32.08 (t_{40}=-1.87, p=0.069) relative to the treatment with no predators (Figure 6). In contrast, the predator assemblage that occurs after exposure to 0.064mg/L TMX enhanced (125.43 +/- 51.26) (111.48 more) the number of egg casings found relative to the treatment with no predators (t_{40}=1.68, p=0.101) (Figure 6). Consequently, the number of egg masses present was substantially lower when the unaltered predator assemblage was present than when the TMX altered predator assemblage was present (t_{40}=-3.41, p=0.002).

The number of egg masses on the sides of the tanks when total predator abundance was reduced but richness was maintained was intermediate to, and not statistically different from, that observed in the treatment with no predators (t_{40}=-0.36, p=0.722) (Figure 6). A reduction in the total abundance of predators while predator species richness was maintained more than doubled the number of egg masses found relative to that observed when the unaltered predator assemblage was present, but this difference was not statistically different from zero (t_{40}=1.51, p=0.139).

On average, the loss of two predator species while maintaining the total abundance of predators, did not alter the number of egg masses present relative to that observed in the presence of the unaltered predator assemblage (t_{40}=0.34, p=0.738) (Figure 6). The effect of losing 2 predator species while maintaining the total abundance of predators, however, depended on which predator species were lost (Figure 6). In general, the loss of 2 predator species in which one of the lost predator species was *Belostoma* resulted in many more egg cases being found in comparison to when the unaltered predator assemblage was present (*Pachydiplax* and *Ranatra* (t_{40}=-3.2, p=0.003); *Erythemis* and *Ranatra* (t_{40}=-3.03, p=0.004)) but was similar to the number observed when no predators were present (*Pachydiplax* and *Erythemis* (t_{40}=0.93, p=0.36);

Pachydiplax and *Ranatra* (t_{40}=1.39, p=0.172); *Erythemis* and *Ranatra* (t_{40}=1.27, p=0.218)) (Figure 6). When *Belostoma* was not one of the lost species, however, reducing predator species richness actually resulted in many fewer egg masses present when compared to the unaltered predator assemblage (*Pachydiplax* and *Belostoma* (t_{40}=2.6, p=0.013); *Erythemis* and *Belostoma* (t_{40}=2.6, p=0.013); *Belostoma* and *Ranatra* (t_{40}=1.83, p=0.075)) and to when no predators were present (*Pachydiplax* and *Belostoma* (t_{40}=-3.69, p=0.001); *Erythemis* and *Belostoma* (t_{40}=-3.69, p=0.001); *Belostoma* and *Ranatra* (t_{40}=-3.38, p=0.002)) (Figure 6).

CHAPTER IV

DISCUSSION

Regardless of the brand or generation of pesticide, insecticides rarely extirpate all species of insects in an ecosystem (Relyea 2005). Because neonicotinoids were developed to target the acetylcholine receptors of invertebrates as opposed to vertebrates, nontarget invertebrates may vary in their vulnerability to TMX depending on the structure and quantity of their acetylcholine receptors (Yamamoto et al. 1995). Laboratory tests may be sufficient to test the direct toxicity of insecticides necessary to kill half of the individuals in acute stress situations, but they do not address potential indirect effects that direct alterations can have on other species in a freshwater community (Fleeger et al. 2002), nor do they present an opportunity to test the effect of insecticides on species in realistic environments over a realistic amount of time (Relyea 2005). In the first experiment I demonstrate that TMX alters the predator assemblage in many ways, and it appears that TMX-induced changes to the predator assemblage has important consequences for prey. There have been similar community-wide studies on the effect of changes in predator assemblages on the assemblage's ability to suppress prey performed with other neonicotinoids like clothianidin (Miles et al. 2017), however no one has examined what aspects of the predator assemblage that changed (changes to predator richness, abundance, and composition) were most responsible for indirect effects on prey resulting from TMX exposure.

As TMX dose increased the amount of species lost from the mesocosm increased curvilinearly, as did the number of total individual predators lost. Species were not all lost equally, however, and the Hemipterans were most vulnerable to TMX (Table 2). Initially I

believed that the *Belostoma* and *Ranatra* would be less vulnerable to TMX because the LC50 values determined for Hemipterans (0.0649mg/L) is higher than that of Odonates (0.0552mg/L) across all neonicotinoids (Morrissey et al 2015). One explanation for this is that TMX readily breaks down into clothianidin, a different neonicotinoid. When exposed to clothianidin Hemipterans (*Belostoma flumineum;* LC50=0.079mg/L) appear to be more vulnerable to the insecticide than Odonates (*Plathemis lydia;* LC50=0.865mg/L, *Anax junius;* LC50=1mg/L, and *Lestes unguiculatus;* LC50=1.245mg/L), although there is no LC50 level data on *Pachydiplax* or *Erythemis* for clothianidin (Miles et al. 2017). It is possible that the TMX broke down into clothianidin relatively quickly, as breakdown is faster with more sunlight, lower turbidity, and higher pH and temperature (MacBean 2010, Sakar et al. 2001). The experiment was performed during the summer, which could have sped up the breakdown process, causing Hemipterans to be more vulnerable than Odonates. My results agree with Miles et al. (2017) in that species of the same order were similar in vulnerability to TMX.

In the presence of predators and no TMX snail per capita growth rate was negative, but increasing the dose caused the per capita growth rate to become positive when predators were present (Figure 4). Previous connections have been made between the input of insecticides and indirect changes within the community. Top-down trophic cascades have been observed in pond-like mesocosms containing cypermethrin when the insecticide was toxic to *Daphnia* and copepods, leading to higher abundances of rotifers, hetertrophic nanoflagellates, phytoplankton, and periphytic algae (Friberg-Jensen et al. 2003). As cypermethrin increased primary consumers decreased and could no longer suppress their prey. When predatory invertebrates were exposed to clothianidin there was also a positive relationship observed between dose and abundance of

prey (Miles et al. 2017). TMX appeared to have no effect on snail per capita growth rate in the absence of predators (Figure 4).

The positive snail per capita rate of growth observed in treatments where predators were exposed to TMX in the first experiment can potentially be explained by both lethal and sublethal effects of TMX on predators, especially after exposure to levels of TMX that *Belostoma* can survive (less than 0.0077mg/L TMX). Imidacloprid has been shown to inhibit the feeding responses of *Gammarus pulex* as well as *Epeorus longimanus*, however *Gammarus pulex* is able to recover after two days, while in 0.005mg/L imidacloprid mayflies were not able to recover to their initial feeding response (Nyman et al. 2013, Alexander et al. 2007)**.** There is a lack of feeding inhibition data on the effect of TMX on aquatic predators, however clothianidin has been shown to reduce the feeding response of *Belostoma* individuals by 62% after exposure to 0.1mg/L of the insecticide (Miles et al. 2017). After the exposure to thiamethoxam, and potentially clothianidin, and given that organisms in the same order appear to be similarly vulnerable to neonicotinoids, results from the first experiment could demonstrate the effect that both direct mortality and sublethal feeding inhibition have on the ability of the predator assemblage to suppress prey. In concentrations above 0.0077mg/L, however, all *Belostoma* are likely to die off in concentrations equal to or above 0.0634mg/L, at which point sublethal effects are irrelevant as *Belostoma* are the most effective predators. When considering the potential impact on biodiversity in freshwater systems we must take into consideration the duration and intensity of runoff events, as individuals may be able to recover in systems where pulses of insecticides are weak and infrequent.

Although characterizing the effects of TMX on communities is important in assessing ecological risk, insecticides are rarely found singly in environmental samples, and modeling

cumulative effects runs the risk of underestimating impacts by not accounting for synergism (Maloney et al. 2018). Sublethal mixtures of both imidacloprid and thiamethoxam caused reductions in emergence of *Chironomus dilutes* greater than predicted additive effects by up to 10% (Maloney et al. 2018). Because neonicotinoids are often found together in a single sample (Morrissey et al. 2015, Hladik and Kolpin 2015), synergistic cumulative effects should be considered when assessing risk of neonicotinoid contamination.

In addition to understanding the lethal and sublethal effects a pulse of TMX has on the ability of a predator assemblage to suppress prey, I also wanted to explore whether the different types of changes that TMX had on the predator assemblage altered the impact of the predator assemblage on prey suppression. It appears that the predators used in my experiment each have very different impacts on snail prey. My results suggest that *Belostoma* is a voracious predator, and their loss from an environment due to selective toxicity has important consequences for a predator assemblage to suppress prey. Treatments in the second experiment with two species, one of which was *Belostoma,* saw significant reductions in both snail survival and reproduction when compared to an assemblage with higher species diversity (4 species). My results are supported by laboratory feeding tests, in which *Belostoma* consumed more *Physa* than all other predators examined. In laboratory tests of feeding rates, *Belostoma flumineum* consumed an average of 68% (+/-3%) of *Physa acuta, Ranatra nigra* consumed an average of 5% (+/-3%) *Physa acuta, Pachydiplax longipennis* consumed an average of 3% (+/-4%) of *Physa acuta,* and *Erythemis simplicicollis* consumed an average of 55% (+/-6%) of *Physa acuta,* and in mesocosm trials *Belostoma* were successful in suppressing snail biomass and density (Turner and Chislock 2007). In a separate feeding trial, one *Belostoma* is able to consume 6 snails in a 12 hour period, making them very voracious predators (Wojdak et al. 2005). It is important to note, however,

that the second experiment does not address the potential sublethal effects TMX has on invertebrate predators.

Although *Ranatra* seemed to be as vulnerable to TMX as *Belostoma,* there is no evidence to suggest that they are important for suppressing snail prey within this predator assemblage. It is possible that *Ranatra* prefer to consume other types of prey, like crustaceans. *R. montezuma* has been shown to be an efficient predator of *Hyalella montezuma* (Runck and Blinn 1992). Prey behavior may also influence the ability of *Ranatra* to capture prey. As sit-and-wait predators they are more proficient at capturing actively swimming prey, as opposed to more sedentary prey (Runck and Davies 1993). This could explain why *Ranatra* did not consume snail prey efficiently.

My data suggest that *Pachydiplax* and *Erythemis* together, or combined with *Ranatra,* (Figure 4) are not proficient in suppressing prey, and a preference for consuming prey of a certain size could be partially responsible. When the species most vulnerable to TMX are removed from the population the composition that is left (2 *Pachydiplax* and 2 *Erythemis*) was not able to suppress prey, even when abundance of each increased to 5 individuals of each species (Figure 4). In laboratory feeding trials *Anax junius* seems to be an important predator of *Physa,* seeing as they consume snails of all size classes, so they are unable to reach size refugia, while *Pantala hymanae* was mostly unable to eat snails over 3mm (Turner and Chislock 2007). While there is no data on the prey size preference for *Pachydiplax* or *Erythemis*, these species are much smaller than *Anax*, but similar to *Pantala hymanae*, so they may share similar prey size preferences as *P. hymanae.* Snails in my experiment could have reached size refugia such that *Pachydiplax* and *Erythemis* could no longer consume them.

My findings provide evidence for *Belostoma* as important individuals for snail prey suppression, as treatments containing at least 2 *Belostoma* individuals suppressed prey more than treatments without *Belostoma* and without predators (Figure 5). Even when compared to treatments with 4 species and 10 individuals, treatments containing only 2 species when one of those species was *Belostoma* consumed more snails, suggesting that increasing species diversity within this assemblage does not necessarily lead to increased prey suppression (Figure 4). A possible explanation for this phenomenon is that treatments containing 4 species only contained 2 *Belostoma* individuals, while treatments containing 2 species with *Belostoma* contained 5 *Belostoma* each. When abundance increased from 2 *Pachydiplax* and 2 *Erythemis* to 5 individuals of each dragonfly species there was no increase in prey suppression or decrease in snail reproduction, suggesting that abundance may only be important for prey suppression when *Belostoma* abundance is increasing (Figures 5 and 6). Prior work has focused on the idea that fish and crayfish are the most important consumers to suppress snail populations (Lodge et al. 1987), but more recent work has challenged this idea (Turner and Chislock 2007; Kesler and Munns 1989). Both *Anax* and *Belostoma* have been shown to significantly reduce snail biomass and population size in mesocosms (Turner and Chislock 2007). Belostoma have also been recorded as heavily altering snail populations and are often indiscriminate in the species and size of snail they consume (Kesler and Munns 1989).

In this assemblage *Belostoma* may be acting as the most efficient snail predator, therefore the results align closely with the finding that a secondary consumer assemblage is only as successful at suppressing prey as the most efficient predator (Griffin et al. 2013). It is important to note that the effect of TMX was only measured for 4 macroinvertebrate predators and one prey species. The presence or absence of keystone predators could affect the competitive

interactions between prey species (Paine 1969). *Belostoma* appear to consume snails even at low *Belostoma* abundances and prevented snails from increasing in abundance, which could classify them as keystone species (Power et al. 1996). Compared to *Ranatra, Pachydiplax,* and *Erythemis, Belostoma* appeared to control snail survival more than other predator species at the same abundance (Figures 4 and 5). If *Belostoma* is indeed acting as a keystone species further research should be performed on the effect of TMX-induced loss of predators on the competitive interactions between multiple prey species.

Although the unaltered assemblage (4 species, 10 individuals) observed in experiment 1, then applied to experiment 2, was capable of suppressing both snail survival and reproduction more than the treatment without predators, treatments with only two species (decreased species richness), one being *Belostoma,* were more successful at suppressing snail survival and reproduction (Figures 5 and 6). Treatments with 2 species where neither species was *Belostoma,* including the composition most likely to be observed after exposure to TMX, was no more successful at suppressing snails than treatments without predators. Increased species richness seems to be less important for prey suppression than the identity of the predators. The assemblage consisting of the species diversity and abundance observed after TMX (2 *Pachydiplax* and *2 Erythemis*) was not proficient at suppressing snails or snail reproduction, and even had higher egg mass counts than the treatment containing no predators (Figures 5 and 6). If the abundances of the species least vulnerable to TMX were allowed to recover there is still evidence to suggest that they would not be able to suppress snails to the same degree as the unaltered predator assemblage, or to the same degree as treatments that contain *Belostoma.* Furthermore, when species richness (4 species) was held constant and abundance decreased (1 individual of each species) the assemblage suppressed prey and prey reproduction to a similar

degree as both the full assemblage and the treatment with no predators. This is again most likely due to a smaller population of *Belostoma,* rather than a loss of abundance of each species.

Although the geometric mean peak surface water contamination worldwide is relatively low, 0.63ug/L, these concentrations only represent snapshots of concentrations at a single point in time, and may underestimate true maximum concentrations (Xing et al. 2013; Morrissey et al. 2015). Neonicotinoid concentrations are variable depending on precipitation, and often peak during spring and fall near areas of agriculture (Struger et al. 2017). It is also important to note that because of routine transport of neonicotinoids from various deposition mechanisms (rainfall and snowmelt), exposure to freshwater environments is likely chronic and occurs even outside the main crop planting period (Wood and Goulson 2017). Concentrations of neonicotinoids are positively correlated with higher proportions of surrounding agriculture (Hladik and Kolpin 2016; Wood and Goulson 2017). Not only that, but as more neonicotinoids are applied as seed coatings as opposed to aerial spraying, the risk of runoff into freshwater environments may also increase (Hladik et al. 2018), however Canada set a goal to reduce the amount of corn and soybean acreage using neonicotinoid seed coatings by 80% (Neonicotinoid regulations 2015). Globally, however, neonicotinoid use is expected to rise, there is a growing need to explore the effect they have on nontarget invertebrates, as well as their indirect impacts as well. Results from my first experiments suggest that TMX has potential lethal and sublethal effects on predators, which reduced the assemblage's ability to suppress the prey population, even at the lowest concentration of TMX, and that Hemipterans, like *Belostoma* and *Ranatra* are more vulnerable in artificial ponds than Odonates. Results from the second experiment highlight that when *Belostoma* is lost from an invertebrate predator assemblage due to their vulnerability to TMX the

ability of the assemblage to suppress prey and prey reproduction is equivalent to having no predators in the system, likely due to rate at which *Belostoma* are able to consume snails.

Results from these experiments demonstrate that intermediate and high levels of TMX reduce the total abundance and the species richness of the predator assemblage (Figures 2 and 3). Furthermore, it caused a change in predator composition by selectively removing the Hemipterans (*Ranatra* and *Belostoma*) from the assemblage (Table 2). Despite all of these changes, it appears that it is the change in which species that survive is the most important way through which thiamethoxam reduces the ability of predators to suppress prey.

In conclusion, the addition of TMX appears to compromise the ability of the predator assemblage to suppress prey. Predator assemblages suppress prey populations in the absence of TMX, but predators have little impact on snail populations when TMX is present. The idea that the ability of a predator assemblage to suppress prey can be compromised by the addition of TMX is not a surprise given that I found that TMX reduced the number of predator species present, reduced the total number of predators present, and altered which predator species will likely be present in an assemblage. Despite all of these changes to the predator assemblage as the result of TMX exposure, it appears that the primary mechanism through which TMX compromises the ability of the predator assemblage to suppress prey is that it kills the most effective predators. Though some have argued that TMX can indirectly affect prey via sublethal mechanisms that operate on predators, it is unlikely that such sublethal effects are important here. This work reveals that the application of pesticides can compromise the ability of natural assemblages of predators to suppress their prey and people need to be cautious about the indirect effects that can result from the application of pesticides to the environment. Future research

should explore the sublethal effects TMX has on important predators, as well as the effect that changes in predator assemblages have on competitive interactions between prey species.

Table 1: Contrasts and coefficients for experiment 2. For "Predators Present," P represents *Pachydiplax*, E represents *Erythemis*, B represents *Belostoma*, and R represents *Ranatra*.

Contrast	Description		Treatment									
			1	2	3	4	5	6	7	8	9	10
		Predators Present	3P, 3E, 2B, 2R	2P, 2E	1P, 1E, 1B, 1R	5P, 5E	5P, 5B	5P, 5R	5E, 5B	5E, 5R	5B, 5R	None
		Predator Species Richness	4	2	4	2	2	2	2	2	2	0
		Predator Abundance	10	4	4	10	10	10	10	10	10	0
		TMX Impact on Predator Assemblage	Unaltered by TMX	Reduction in richness and abundance	Reduced abundance, all species vulnerable	2 most vulnerable species are lost	2 species lost, not most vulnerable to TMX	2 species lost, not most vulnerable to TMX	2 species lost, not most vulnerable to TMX	2 species lost, not most vulnerable to TMX	2 species lost, not most vulnerable to TMX	
1	Does an unaltered predator assemblage affect prey?		1									-1
2	Is the predator assemblage still able to suppress prey after TMX?			1								-1
3	How does the effect of the altered predator assemblage differ from the unaltered assemblage?		1	-1								
4	Does the predator assemblage with fewer predators suppress prey?				1							-1
5	How does a decline in abundance affect influence of the assemblage?		1		-1							
6	How does a decline in richness alter influence of predator assemblage?		6			-1	-1	-1	-1	-1	-1	

7	How does a TMX-induced decline in richness affect influence of the assemblage?	1		-1								
8a	How do different predator compositions vary in their ability to suppress prey?			1								-1
8b					1							-1
8c						1						-1
8d							1					-1
8e								1				-1
8f									1			-1
9a	How does a decline in richness and change in composition affect the ability of the assemblage to suppress prey when compared to a full assemblage?	1			-1							
9b		1				-1						
9c		1					-1					
9d		1						-1				
9e		1							-1			

Table 2: Least square mean (+/- 1 SE) number of individuals lost for each predator species from mesoscosms that differ in the amount of TMX present. 6 individuals of each predator species was present at the start of the experiment.

TMX Conc. (mg/L)	*Belostoma*	*Ranatra*	*Erythemis*	*Pachydiplax*
0	4 +/-0.29	3.2 +/- 0.35	3.8 +/- 0.70	3.8 +/-0.48
0.0077	5 +/-0.29	5.4 +/-0.35	2.6 +/-0.70	2.6 +/- 0.48
0.0634	5.6 +/- 0.29	6 +/-0.35	4.2 +/-0.70	4.4 +/-0.48
0.225	6 +/-0.29	6 +/-0.35	5.2 +/-0.70	5.6 +/-0.48

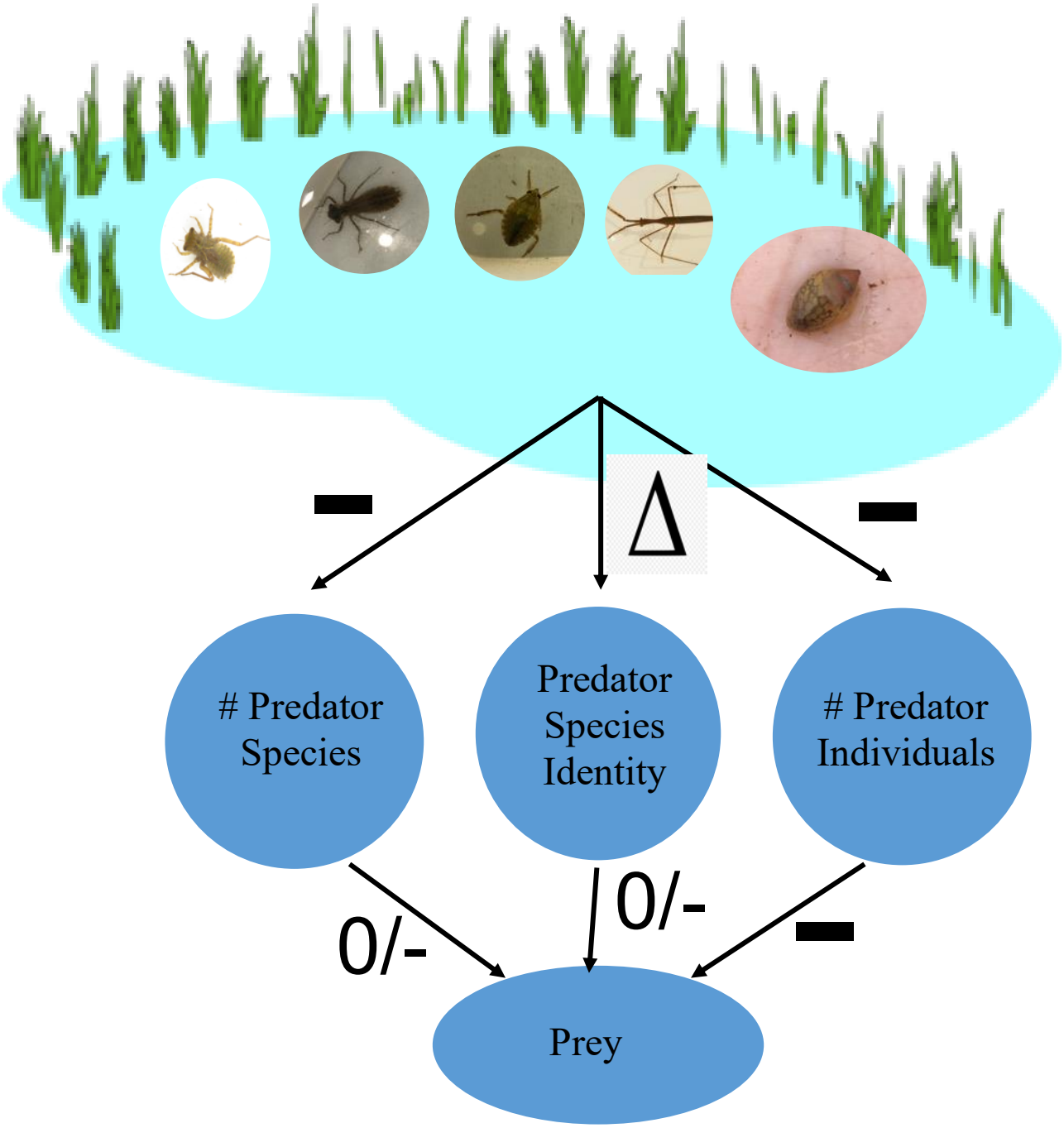

Figure 1: Diagram showing hypothesized impacts of TMX on the predator assemblage and its ability to suppress prey. It was predicted that TMX would reduce the number of predator individuals (-), reduce the number of predator species (-), and alter the predator composition (Δ). I also predicted that reducing the total abundance of predator individuals would have a negative impact on the ability of the predator assemblage to suppress prey (-), reducing the number of predator species or changing species composition would have no effect on the ability of the assemblage to suppress prey or would have a negative impact on the ability of the predator assemblage to suppress prey (0/-).

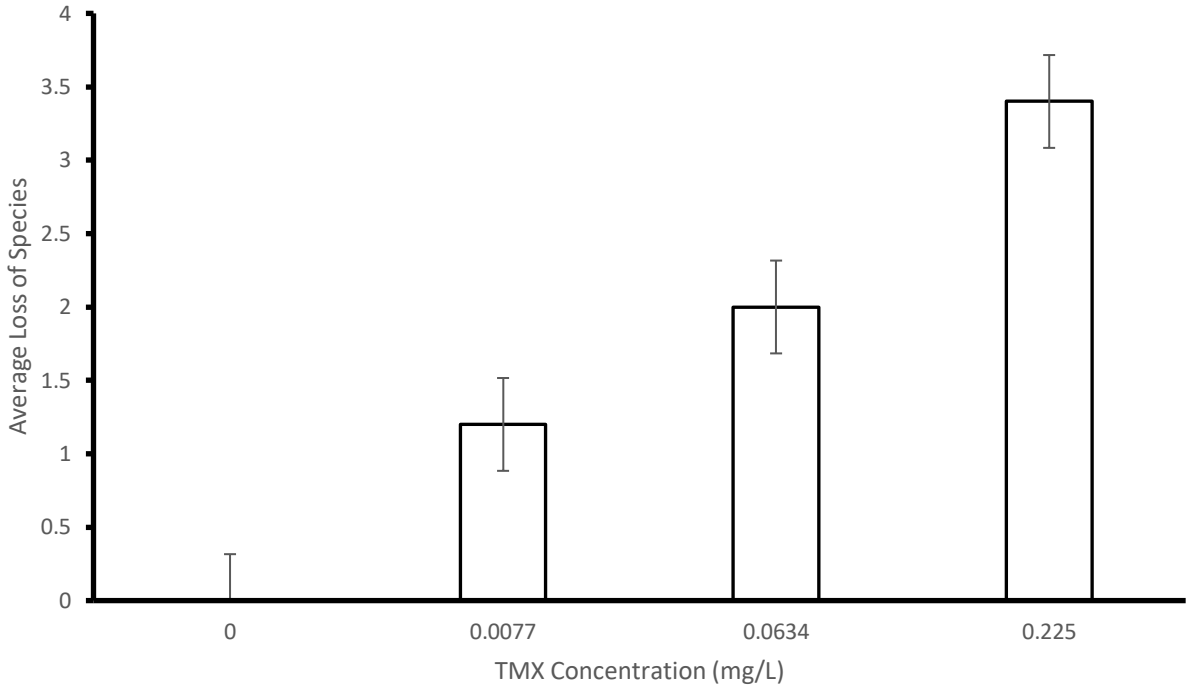

Figure 2: Least square mean (+/-1 SE) number of species lost after exposure to each level of TMX.

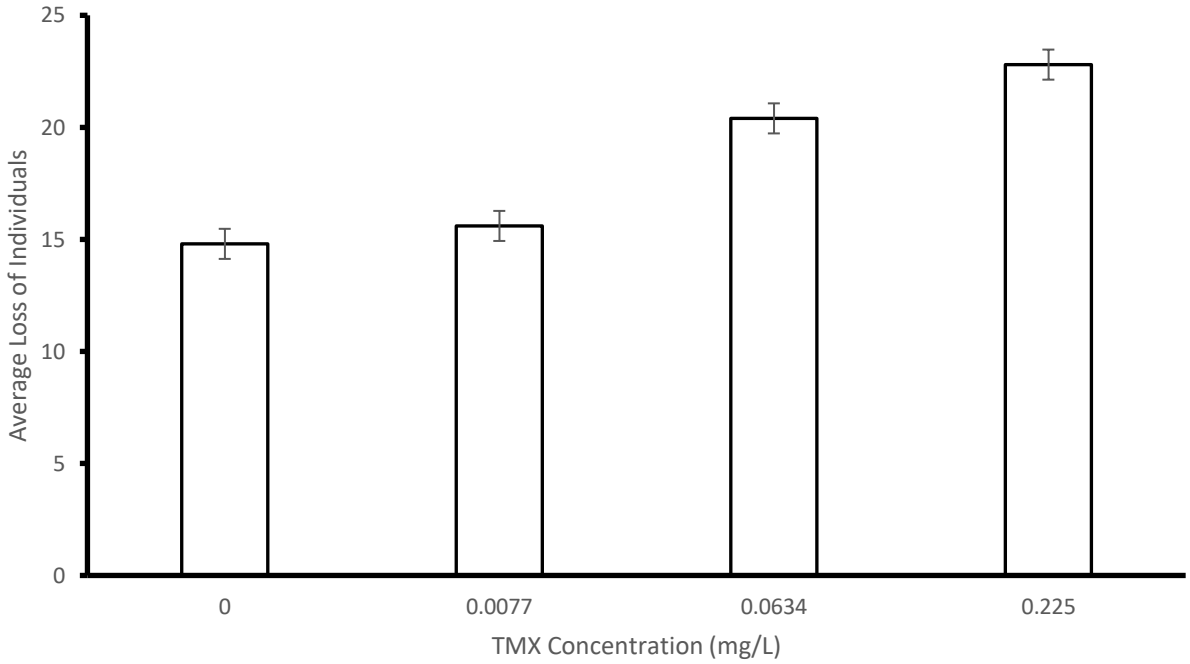

Figure 3: Least square mean (+/-1 SE) number of individuals lost after exposure to each level of TMX.

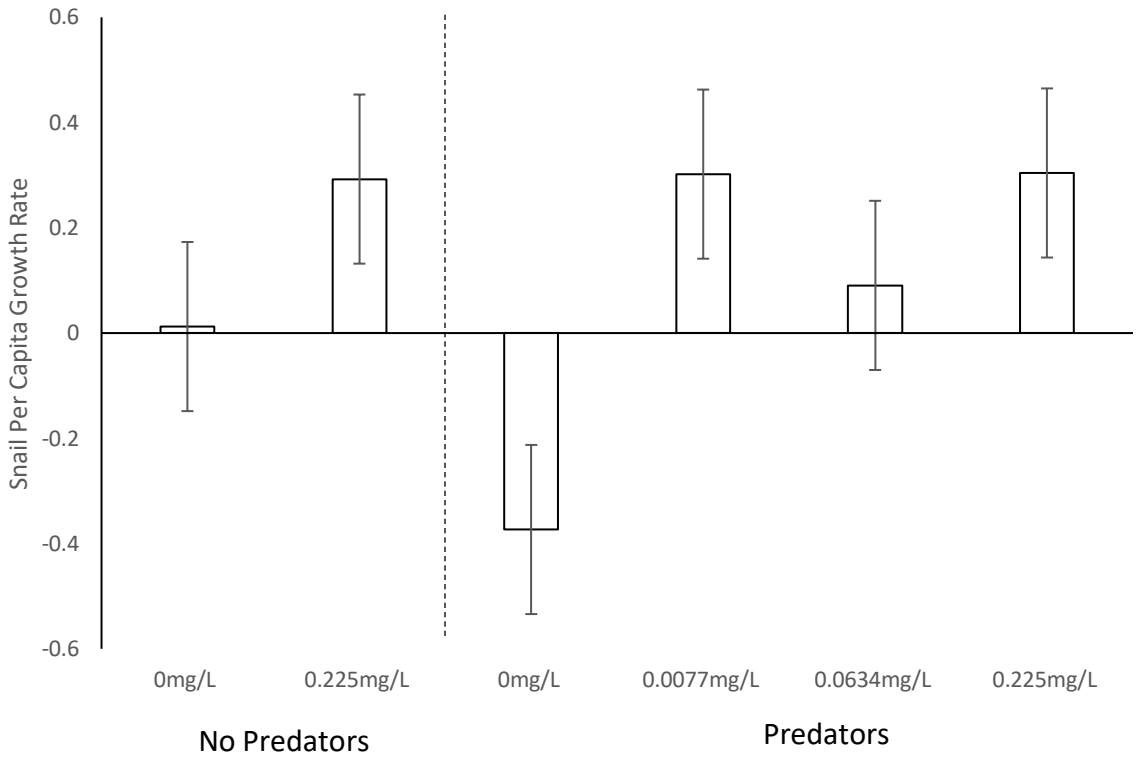

Figure 4: Least square mean (+/-1 SE) estimates of the per capita rate of population growth for snails in tanks with or without predators and varying levels of TMX.

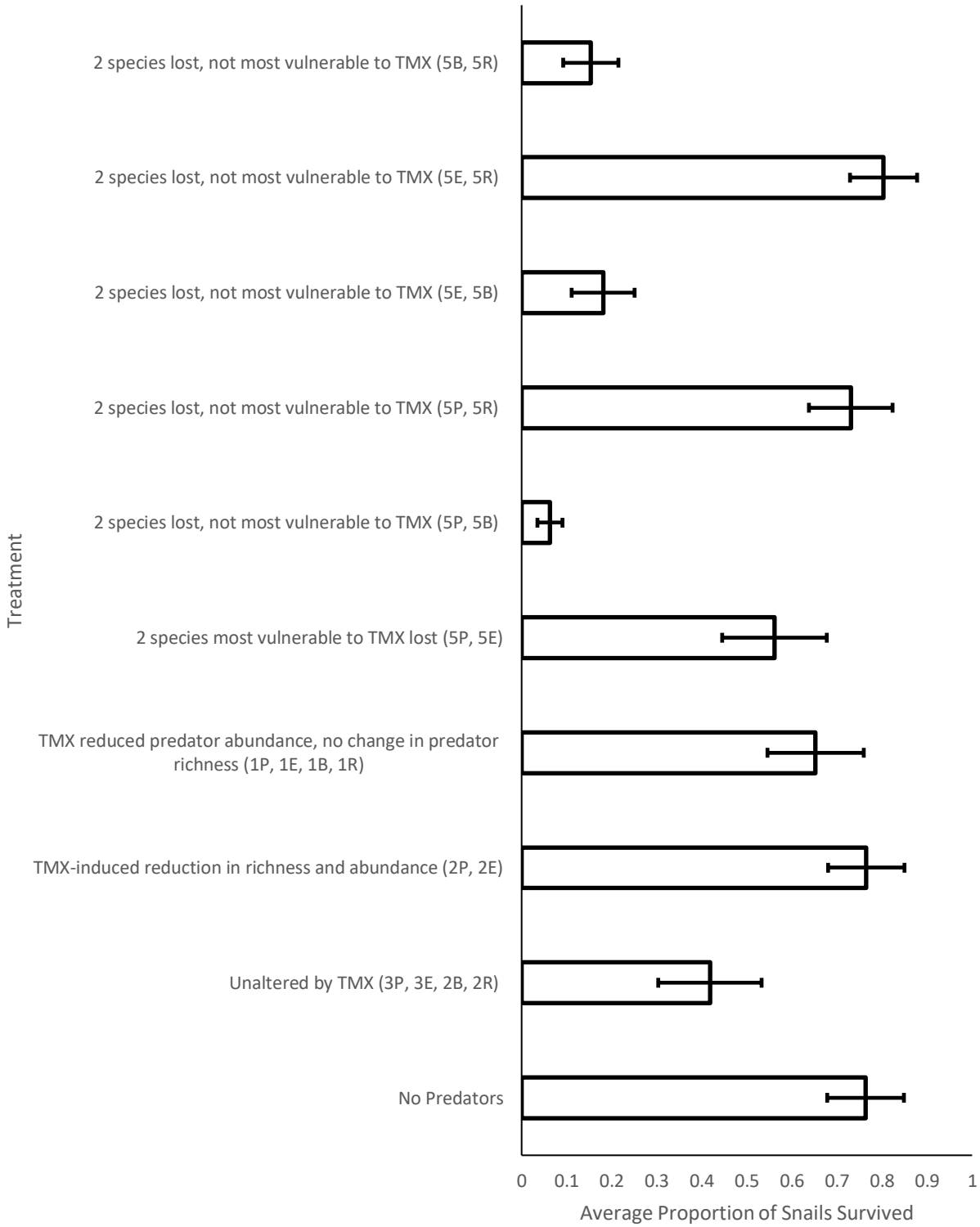

Figure 5: Mean (+/-1 SE) proportion of snails that survived until the end of the experiment. P represents *Pachydiplax*, E represents *Erythemis*, B represents *Belostoma*, and R represents *Ranatra*. The numbers in treatment before the species represents the number of individuals of that species.

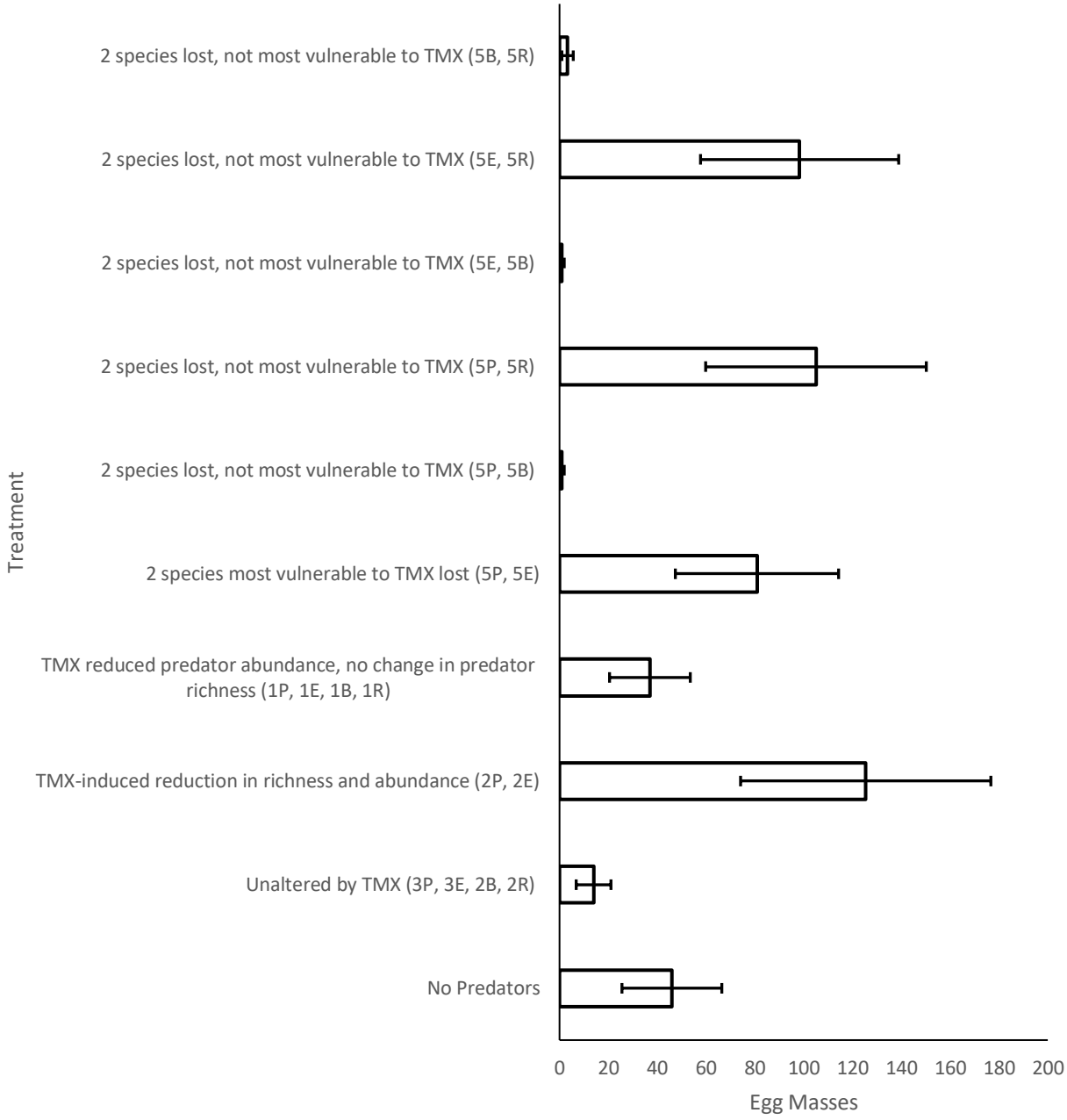

Figure 6: Mean (+/-1 SE) number of egg casings laid by snails on the wall of the cattle tank. P represents *Pachydiplax*, E represents *Erythemis*, B represents *Belostoma*, and R represents *Ranatra*. The numbers in treatment before the species represents the number of individuals of that species.

CHAPTER V

REFERENCES

Actara Product Label. Syngenta Seed Protection.

Alexander, A. C., J. M. Culp, K. Liber, and A. J. Cessna. 2007. Effects of insecticide exposure on feeding inhibition in mayflies and oligochaetes.*Environmental Toxicology and Chemistry* 26:1726.

Anderson, T. A., C. J. Salice, R. A. Erickson, S. T. Mcmurry, S. B. Cox, and L. M. Smith. 2013. Effects of landuse and precipitation on pesticides and water quality in playa lakes of the southern high plains. *Chemosphere* 92:84-90.

Ashauer, R. A. Agatz, C. Albert, V. Ducrot, N. Galic, J. Hendriks, T. Jager, A. Dretschmann, I. O'Conner, M. N. Rubach, A. Nyman, W. Schmitt, J. Stadnicka, P.J. van den Brink, T.G. Preuss. 2011. *Environmental Toxicology and Chemistry* 30(11): 2519-2524.

Chalcraft, D. R., and W. J. Resetarits. 2003. Predator Identity and Ecological Impacts: Functional Redundancy or Functional Diversity? *Ecology* 84:2407-2418.

Conley, D. J., H. W. Paerl, R. W. Howarth, D. F. Boesch, S. P. Seitzinger, K. E. Havens, C. Lancelot, and G. E. Likens. 2009. Controlling Eutrophication: Nitrogen and Phosphorus. *Science* 323:1014–1015.

Fleeger, J. W., K. R. Carman, and R. M. Nisbet. 2003. Indirect effects of contaminants in aquatic ecosystems. *The Science of the Total Environment* 317:207–233.

Friberg-Jensen, U., L. Wendt-Rasch, P. Woin, and K. Christoffersen. 2003. Effects of the pyrethroid insecticide, cypermethrin, on a freshwater community studied under field conditions. I. Direct and indirect effects on abundance measures of organisms at different trophic levels. *Aquatic toxicology* 63:357–71.

Gill, R. J., O. Ramos-Rodriguez, and N. E. Raine. 2012. Combined pesticide exposure severely effects individual-and colony-level traits in bees. *Nature* 491: 105-109.

Griffin, J.N., J.E.K. Byrnes, B.J. Cardinale. 2013. Effects of predator richness on prey suppression: a meta-analysis. *Ecology* 94(10): 2180-2187.

Hallmann, C.A., R.P.B. Foppen, C.A.M. van Turnhout, H. de Kroon, E. Jongejans. 2014. Declines in insectivorous birds are associated with high neonicotinoid concentrations. *Nature* 511: 341-343

Jeschke, P., and R. Nauen. 2008. Neonicotinoids-from zero to hero in insecticide chemistry. *Pest Management Science* 64:1084–1098.

Kessler, S.C., E.J. Tiedeken, K. L. Simcock, S. Derveau, J. Mitchell, S. Softley, A. Radcliffe, J.C. Stout, G.A. Wright. 2015. Bees prefer foods containing neonicotinoid pesticides. *Nature* 521: 74-76.

Kurzava, L. M., and P. J. Morin. 1998. Tests of Functional Equivalence: Complementary Roles of Salamanders and Fish in Community Organization. *Ecology* 79:477-489.

Lodge, D., K. Brown, S. Klosiewski, R. Stein, A. Covich, B. Leathers, and C. Bronmark. 1987. Distribution of freshwater snails: spatial scale and the relative importance of physicochemical and biotic factors. *American Malacological Bulletin* 5:73-84.

MacBean, C. 2010. The e-Pesticide Manual. 15th edition. Version 5.0.1. Surrey UK. British Crop Protection Council.

Maloney, E. M., C. A. Morrissey, J. V. Headley, K. M. Peru, and K. Liber. 2018. Can chronic exposure to imidacloprid, clothianidin, and thiamethoxam mixtures exert greater than additive toxicity in Chironomus dilutus? *Ecotoxicology and Environmental Safety* 156:354–365.

McKnight, U. S., J. J. Rasmussen, B. Kronvang, P. J. Binning, and P. L. Bjerg. 2015. Sources, occurrence and predicted aquatic impact of legacy and contemporary pesticides in streams. *Environmental Pollution* 200:64–76.

Miles, J. C., J. Hua, M. S. Sepulveda, C. H. Krupke, and J. T. Hoverman. 2017. Effects of clothianidin on aquatic communities: Evaluating the impacts of lethal and sublethal exposure to neonicotinoids. *PLoS ONE* 12:1–24.

Morrissey, C. A., P. Mineau, J. H. Devries, F. Sanchez-Bayo, M. Liess, M. C. Cavallaro, and K. Liber. 2015. Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: A review. *Environment International* 74:291–303.

Neonicotinoid regulations | Ontario.ca. 2015. . https://www.ontario.ca/page/neonicotinoid-regulations.

Nyman, A. M., A. Hintermeister, K. Schirmer, and R. Ashauer. 2013. The Insecticide Imidacloprid Causes Mortality of the Freshwater Amphipod Gammarus pulex by Interfering with Feeding Behavior. *PLoS ONE* 8:e62472.

Özdemir, S., S. Altun, M. Özkaraca, A. Ghosi, E. Toraman, and H. Arslan. 2018. Cypermethrin, chlorpyrifos, deltamethrin, and imidacloprid exposure up-regulates the mRNA and protein levels of bdnf and c-fos in the brain of adult zebrafish (*Danio rerio*). *Chemosphere* 203:318–326.

Pace, Cole, Carpenter, and Kitchell. 1999. Trophic cascades revealed in diverse ecosystems. *Trends in ecology & evolution* 14:483–488.

Paine, R. T. 1969. The Pisaster-Tegula Interaction: Prey Patches, Predator Food Preference, and Intertidal Community Structure. *Ecology* 50:950–961.

Pisa, L. W., V. Amaral-Rogers, L. P. Belzunces, J. M. Bonmatin, C. A. Downs, D. Goulson, D. P. Kreutzweiser, C. Krupke, M. Liess, M. Mcfield, C. A. Morrissey, D. A. Noome, J. Settele, N. Simon-Delso, J. D. Stark, J. P. Van Der Sluijs, H. Van Dyck, and M. Wiemers. 2014. Effects of neonicotinoids and fipronil on non-target invertebrates. Environmental *Science and Pollution Research* 22:68–102.

Polis, G. A., C. A. Myers, and R. D. Holt. 1989. The Ecology and Evolution of Intraguild Predation: Potential Competitors That Eat Each Other. *Annual Review of Ecology and Systematics* 20:297–330.

Robinson, S. A., S. D. Richardson, R. L. Dalton, F. Maisonneuve, V. L. Trudeau, B. D. Pauli, and S. S. Y. Lee-Jenkins. 2017. Sublethal effects on wood frogs chronically exposed to environmentally relevant concentrations of two neonicotinoid insecticides. *Environmental Toxicology and Chemistry* 36:1101–1109.

Roessink, I., L.B. Merga, H.J. Zweers, P.J. Van den Brink. 2013. The neonicotinoid imidacloprid shows high chronic toxicity to mayfly nymphs. *Environmental Toxicology and Chemistry* 32: 1096–1100.

Rohr, J. R., and P. W. Crumrine. 2005. Effects of an herbicide and an insecticide on pond community structure and processes. *Ecological Applications* 15:1135–1147.

Samson-Robert, O., G. Labrie, M. Chagnon, and V. Fournier. 2014. Neonicotinoid-Contaminated Puddles of Water Represent a Risk of Intoxication for Honey Bees. *PLoS ONE* 9:e108443.

Sarkar, M.A., S. Roy, R.K. Kole, A. Chowdhury. 2001. Persistence and metabolism of imidacloprid in different soils of West Bengal. *Pest Management Science* 57: 598–602.

SAS. 2010 SAS version 9.0 SAS, Cary, NC.

Simon-Delso, N., V. Amaral-Rogers, L. P. Belzunces, J. M. Bonmatin, M. Chagnon, C. Downs, L. Furlan, D. W. Gibbons, C. Giorio, V. Girolami, D. Goulson, D. P. Kreutzweiser, C. H. Krupke, M. Liess, E. Long, M. McField, P. Mineau, E. A. D. Mitchell, C. A. Morrissey, D. A. Noome, L. Pisa, J. Settele, J. D. Stark, A. Tapparo, H. Van Dyck, J. Van Praagh, J. P. Van der Sluijs, P. R. Whitehorn, and M. Wiemers. 2015. Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. *Environmental Science and Pollution Research* 22:5–34.

Soluk, D. A. 1993. Multiple Predator Effects: Predicting Combined Functional Response of

Stream Fish and Invertebrate Predators. *Ecology* 74:219–225.

Swisher, B. J., D. A. Soluk, and D. H. Wahl. 2006. Non-Additive Predation in Littoral Habitats: Influences of Habitat Complexity. *Oikos* 81:30-37.

Tomizawa, M., and J. E. Casida. 2003. Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. *Annual review of entomology* 48:339–64.

United States Department of Agriculture (USDA) - National Agriculture Statistics Service (NASS)

US Geological Survey. Pesticide National Synthesis Project. 2014. Pesticide Use Maps Thiamethoxam 2011-2015.

Van Dijk, T. C., M. A. Van Staalduinen, and J. P. Van der Sluijs. 2013. Macro-Invertebrate Decline in Surface Water Polluted with Imidacloprid. *PLoS ONE* 8:e62374.

Vance-Chalcraft, H. D., J. A. Rosenheim, J. R. Vonesh, C. W. Osenberg, and A. Sih. 2007. The influence of intraguild predation on prey suppression and prey release: a meta-analyses. *Ecology* 88:2689–2696.

Vance-Chalcraft, H. D., and D. A. Soluk. 2005. International Association for Ecology Estimating the Prevalence and Strength of Non-Independent Predator Effects. *Oecologia* 146:452–460.

Vogel, J. R., M. S. Majewski, and P. D. Capel. 2008. Pesticides in Rain in Four Agricultural Watersheds in the United States. *Journal of Environment Quality* 37:1101.

Wang, Y., Y. Zhang, W. Li, Y. Han, and B. Guo. 2019. Study on neurotoxicity of dinotefuran, thiamethoxam and imidacloprid against Chinese lizards (*Eremias argus*). *Chemosphere* 217:150–157.

Wojdak, J. M., B. Luttbeg, and L. Persson. 2005. Relative Strengths of Trait-Mediated and Density-Mediated Indirect Effects of a Predator Vary with Resource Levels in a Freshwater Food Chain. *Oikos* 111: 592-598.

Wood, T. J., and D. Goulson. 2017. The environmental risks of neonicotinoid pesticides: a review of the evidence post 2013. *Environmental science and pollution research international* 24:17285–17325.

Yamamoto, I., G. Yabuta, M. Tomizawa, T. Saito, T. Miyamoto, and S. Kagabu. 1995. Molecular Mechanism for Selective Toxicity of Nicotinoids and Neonicotinoids. *Journal of Pesticide Science* 20:33–40.

Xing, Z., L. Chow, H. Rees, F. Meng, S. Li, B. Ernst, G. Benoy, T. Zha, and L. M. Hewitt. 2013.

Influences of Sampling Methodologies on Pesticide-Residue Detection in Stream Water. *Archives of Environmental Contamination and Toxicology* 64:208–218.