

ABSTRACT

ALICEA, ANNA BARNES. Assessing the Transport, Fate and Effects of Agricultural Herbicides in the South Atlantic Coastal Plain (Under the direction of Dr. W. Gregory Cope).

In the estuaries of the United States, particularly southeast Atlantic and Gulf coasts, submerged aquatic vegetation (SAV) are an important component of a thriving aquatic ecosystem. SAV are a unique group of plants that have adapted to exist fully submerged in aquatic ecosystems. SAV influence the physical, chemical, and biological environments and serve important ecological roles in aquatic ecosystems while providing high value ecosystem services. Submerged aquatic vegetation are one of North Carolina's most valuable aquatic resources and a single acre of grasses can support as many as 40,000 fish and 50 million small invertebrates.

SAV are a class of aquatic plants that are often subject to stress and are often used as one of the five sensitive indicators of pollution in the U.S. National Estuarine Eutrophication Assessment. SAV declines have been documented all over the world and are often being diminished at a concerning rate. All SAV species in Chesapeake Bay declined substantially in the late 1960s and 1970s and were correlated with increasing nutrient and sedimentation from the surrounding watershed.

Lake Mattamuskeet, located within the Albemarle-Pamlico Estuarine System of North Carolina, serves as critical habitat for overwintering waterfowl populations and supports a productive recreational fishery. Lake Mattamuskeet has incurred SAV declines since 1989 with its eventual extirpation in 2015 from the lake to date. There are many potential factors that have contributed to this situation such as water clarity, water depth, excess nutrients, and sediment influx and composition. Among the many threats to SAV, the issue of herbicide exposure and

toxic effects is important due to the extensive agricultural production that occurs in the surrounding drainage areas.

Agricultural pesticide runoff, atmospheric deposition, spray drift, and drainage influx are all recognized routes of potential contamination in aquatic ecosystems. Small water bodies are most susceptible to higher inputs of agricultural chemicals given their proximity to adjacent agricultural fields and lower water volume and discharge. Relevant pesticide parent and metabolite residues from runoff, drift or erosion may elicit a toxic response to organisms and vascular plants in aquatic ecosystems. Several estuaries along the southeast Atlantic and Gulf coasts of the United States are surrounded by agricultural lands, which increases the likelihood of pesticide runoff, erosion or drift. Herbicides have commonly been detected in several estuarine environments in South Florida and in the Gulf of Mexico with the most commonly detected herbicides being metolachlor, atrazine, simazine, cyanazine, and alachlor.

The overall goal of this dissertation was to determine whether restoration efforts for SAV at Lake Mattamuskeet would be adversely affected by residues of agricultural herbicide runoff from the surrounding watershed. This study used a comprehensive hazard and risk assessment framework to assess the potential adverse effects of agricultural herbicides on SAV, integrating herbicide use data, crop type and acreage, modeling, and empirical measurements of herbicides in surface water of Lake Mattamuskeet. In the final retrospective risk assessment, maximum modeled predicted surface water concentrations of herbicides and time weighted average surface water herbicide concentrations from passive samplers were compared to toxicity data from scientific literature. Results indicated that targeted herbicides pose little to no risk to SAV restoration efforts at Lake Mattamuskeet.

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Assessing the Transport, Fate and Effects of Agricultural Herbicides in the South Atlantic
Coastal Plain

by
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DEDICATION

This dissertation is dedicated to my charming husband, Edwin Alicea, without whose support and patience I could not have completed this degree.

BIOGRAPHY

Anna Barnes was born in Selma, North Carolina with her mother, Angela, father Michael, and siblings. Anna always had a passion for learning and graduated third in her class from North Johnston High School in Kenly, North Carolina. After high school, Anna completed a four-year degree in three years in Environmental Technology with a minor in Environmental Toxicology. After graduation in 2010, Anna entered a master's program in Environmental Assessment and completed the program in 2012. Anna spent several years with the federal government and private industry. Anna decided to pursue a doctorate degree in 2016 to further her career and open more doors as an agriculture professional.

In her free time, Anna enjoys travelling and spending time with her family, including her four-legged sons, husband, parents, siblings, nieces and nephew.

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CHAPTER 1: Introduction

The presence of a large number of pesticides in surface water has been well documented around the world (Konstantinou et al., 2005; Geronimo et al., 2014; Fadaei et al., 2012; Palma et al., 2014) with pesticide concentrations being higher in small catchments with intensive agriculture (Lorenz et al., 2017). Agricultural pesticide runoff, atmospheric deposition, spray drift, and drainage influx are all recognized routes of potential contamination in aquatic ecosystems (Lorenz et al., 2017). Small water bodies are most susceptible to higher inputs of agricultural chemicals given their proximity to adjacent agricultural fields and lower water volume and discharge (Lorenz et al., 2017). Moreover, herbicide-monitoring studies have confirmed contamination of reservoirs (Thurman et al., 1996), lakes (Senseman et al., 1997; Thurman et al., 2000), streams (Scribner et al., 2000; Senseman et al., 1997), and rivers (Clark and Goolsby 2000; Senseman et al., 1997; Thurman et al., 1996).

Watershed characteristics can greatly influence pesticide off-target movement potential including soil properties, land use, vegetation cover and topography. Both pesticides in solution and pesticides sorbed to sediment particles can be transported during a runoff event (Holvoet et al., 2007). Movement of pesticides into deep groundwater and possible input of pesticides into surface waters by outflowing groundwater resources is assumed to be negligible, as pesticides in groundwater generally occur at relatively low concentrations (Holvoet et al., 2007).

Relevant pesticide parent and metabolite residues from runoff, drift or erosion may elicit a toxic response to aquatic plants in aquatic ecosystems. Liess and Ohe (2004) studied patterns in aquatic invertebrate community composition and related to the effects of pesticides. Their results demonstrated that concentrations of 1/10 of the acute 48-hour median lethal concentration (LC) of *Daphnia magna* led to short- and long-term reduction of abundance and number of

species at risk. Concentrations of 1/100 of the acute 48-hour LC 50 of *Daphnia magna* correlated with long term change of community composition (Liess and Ohe, 2004).

Several estuaries along the southeast Atlantic and Gulf coasts of the United States are surrounded by agricultural lands, which increases the likelihood of pesticide runoff, erosion or drift. Herbicides have commonly been detected in several estuarine environments in South Florida and in the Gulf of Mexico (Lewis et al., 2001) with the most commonly detected herbicides being metolachlor, atrazine, simazine, cyanazine, and alachlor (Clark and Goolsby 2000; Scribner et al., 2000; Senseman et al., 1997; Thurman et al., 1996). Concentrations of atrazine were linked to sugarcane production near coastal bays and estuaries in Louisiana (Southwick et al., 2002). Powell et al., (2017) found measured concentrations of alachlor, atrazine and metolachlor with mean concentrations ranging from 0.029 to 2.46 $\mu\text{g/L}$ for alachlor, 0.014 to 7.17 $\mu\text{g/L}$ for atrazine, and 0.017 to 5.87 $\mu\text{g/L}$ for metolachlor in the Albemarle Pamlico Estuarine System (APES) region of North Carolina. Concentrations of alachlor, atrazine, and metolachlor measured in water samples from the APES region in 2000 exceeded several of the established benchmarks, standards, or guidelines for protection of aquatic plants (Powell et al., 2017).

Submerged aquatic vegetation (SAV) are a unique group of plants that have adapted to exist fully submerged in aquatic ecosystems. SAV influence the physical, chemical, and biological environments and serve important ecological roles in aquatic ecosystems while providing high value ecosystem services (Costanza et al., 1997). SAV utilize carbon dioxide and inorganic nutrients to transfer the sun's energy into carbohydrates and proteins (Stevenson et al., 1979). This process is critical as it forms the basis for food webs in aquatic ecosystems.

While producing large quantities of organic carbon and stabilizing sediments, SAV provides critical habitat, food, and cover for many waterfowl and fish species of management priority (Orth et al., 2006). Many duck, fish, shrimp and snail species graze on SAV while other species filter SAV-derived detritus from water and sediments to obtain nutrients (Stevenson et al., 1979). SAV provides spawning fishes and their offspring with protection from predators. Juvenile stages of economically important species of fish are also able to utilize SAV as a nursery ground (Stevenson et al., 1979). The proximity of SAV to other beds of critical habitat facilitates trophic transfers and cross-habitat utilization by fish and invertebrates (Beck et al., 2001).

SAV are a class of aquatic plants that are often subject to stress and are often used as one of the five sensitive indicators of pollution in the U.S. National Estuarine Eutrophication Assessment (Bricker et al., 2003). SAV declines have been documented all over the world and are often being diminished at a concerning rate. All SAV species in Chesapeake Bay declined substantially in the late 1960s and 1970s (Orth and Moore, 1983) and were correlated with increasing nutrient and sedimentation from the surrounding watershed. During the 1950s, eel grass beds began to diminish in Japan and their subsequent disappearance resulted in lack of nutrient control, habitat and food sources for commercial fish (Yamamuro et al., 2006). The authors also suggested that 2,4-D herbicide usage may have been the cause for the loss of SAV (Yamamuro et al., 2006). In Florida, the St. Lucie Estuary and Indian River Lagoons have demonstrated significant declines of SAV and the declines have contributed to altered estuarine salinity, increased nutrients and dissolved organic matter and increased epiphyte growth (Sime, 2005). In contrast, SAV expansion has been reported in the Sacramento-San Joaquin River Delta and has greatly improved water quality by decreasing turbidity in the water (Hestir et al., 2016).

Powell et al., (2017) found supporting evidence relating herbicide transport and measurement in surface water to timing of application and that herbicides are present in the surface water when SAV are actively growing. Very few studies have been performed that relate SAV decline to the agricultural use of herbicides in nearby watersheds. Several possible explanations for the lack of studies on herbicides may be related to situations where water quality is already poor due to excess nutrients and sedimentation or the adverse effects from herbicide residues to SAV may be less of a concern (Moorman et al., 2017; Hurley 1991). In the Chesapeake Bay, for example, the effects of herbicides were not likely to be the cause of SAV declines due to poor water clarity due to nutrients and sediment (Hurley 1991). Moreover, larger-scale pesticide studies can be quite costly to conduct.

Lake Mattamuskeet, located within the Albemarle-Pamlico Estuarine System of North Carolina, serves as critical habitat for overwintering waterfowl populations and supports a productive recreational fishery (Moorman et al., 2017). With the exception of Florida, North Carolina has the most extensive acreage of SAV of any state along the Atlantic coast with approximately 99% of SAV found in the Albemarle-Pamlico Estuary (Powell et al., 2017). The North Carolina Division of Marine Fisheries (NCDMF) estimated that between 54,228 to 80,937 hectares of SAV occur along the coast of North Carolina (NCDMF 2015).

Lake Mattamuskeet has incurred SAV declines since 1989 with its eventual extirpation occurring in 2015 from the lake (Moorman et al. 2017). There are many potential factors that have contributed to this situation such as water clarity, water depth, and sediment influx and composition. Among the many threats to SAV, the issue of herbicide exposure and toxic effects is important to examine due to the extensive agriculture production that occurs in the

surrounding drainage areas. Herbicides that potentially enter adjacent waterways through agricultural off-target movement can adversely impact nontarget species such as SAV.

The overall aim of this research was to determine whether restoration efforts for SAV at Lake Mattamuskeet would be adversely affected by residues of agricultural herbicide off-target movement from the surrounding watershed. This study used a comprehensive hazard and risk assessment framework to assess the potential adverse effects of agricultural herbicides on SAV, integrating herbicide use data, crop type and acreage, modeling, and empirical measurements of herbicides in surface water of Lake Mattamuskeet.

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**CHAPTER 2: Modeling the Transport and Fate of Agricultural Herbicides in the Tar-
Pamlico River Basin of North Carolina**

ABSTRACT

Offsite movement of pesticides from applied agricultural areas has been recognized as one of the major contributors to the contamination of surface waters worldwide. The ability to predict contamination concentrations and subsequent restoration effects is critical in designing effective management strategies. Mathematical models that predict exposure to pesticides have been increasingly used in addition to in-situ water quality monitoring to assess the risks of herbicides to organisms, submerged aquatic vegetation, and other components of aquatic ecosystems. For assessing pesticide risks to aquatic organisms, the U.S. Environmental Protection Agency (US EPA) Office of Pesticide Programs (OPP) models pesticide spray drift, runoff and erosion into a hypothetical agricultural pond with specific water body and watershed characteristics using the Pesticide in Water Calculator (PWC). The purpose of this retrospective analysis is to estimate concentrations of targeted herbicides in Lake Mattamuskeet from its surrounding drainage areas in Hyde County, North Carolina due to herbicide movement and shifts in crop type and planted acres. Corn and soybean acres have significantly increased over time ($R^2 = 0.59$ in corn and $R^2 = 0.60$ in soybean) while cotton acres have shown a steady decrease ($R^2 = 0.55$).

Based on PWC results for targeted herbicides, US EPA OPP Aquatic Life Benchmarks and current herbicides of concern in the published literature, several herbicides may have the potential to inhibit SAV restoration at Lake Mattamuskeet. The model results show most significant exposures for atrazine, mesosulfuron, simazine, trifloxysulfuron, cloransulam, pyriithiobac, cyanazine, prometryn and alachlor. A forty-day modeling window was selected to align with field sampling efforts during the 2018 growing season. Comparisons of modeled forty-day pond concentrations demonstrate exceedances in US EPA OPP Aquatic Life

Benchmark concentrations for aquatic plants indicating risk for surface water off-target movement and SAV exposure. Limitations of utilizing the US EPA OPP Aquatic Life Benchmark concentrations exist in that acute studies (<10 days) are represented while the concentrations derived from modeling results are representative of a forty-day window.

Based on the total planted acres in Hyde County for 2018, applications of herbicides to corn and soybeans are of particular concern. In corn, atrazine and metolachlor have the greatest forty-day pond concentrations whereas soybeans have greater forty-day pond concentrations with pyriithiobac. Surface water monitoring for these and other herbicides will be needed to assess and confirm these modeled predictions.

INTRODUCTION

Off-site movement of pesticides from applied agricultural areas has been recognized as one of the major contributors to the contamination of surface waters worldwide (Giannouli and Antonopoulos 2015). A variety of mitigation efforts (such as windbreaks and vegetated buffers) can be utilized, thereby reducing the potential to move into sediment, water, and vegetated ditches. Understanding the potential pathways of transport is essential to the mitigation of the negative impacts on organisms and submerged aquatic plants. The ability to predict pesticide concentrations and subsequent restoration effects should be incorporated into any management plan or subsequent management strategy.

To assess the risks of herbicides to submerged aquatic vegetation from residues in surface water, mathematical models that predict exposure have been increasingly used, in addition to in-situ water quality monitoring (Giannouli and Antonopoulos 2015). Estimated environmental concentrations are commonly regarded as “worst-case” chemical exposure scenarios in freshwater systems by the US EPA (Giannouli and Antonopoulos 2015). A variety of models have been developed to simulate physical, chemical, and microbial processes and predict the fate of pesticides in receiving water bodies (Holvoet et al., 2007). MACRO is a commonly used model and simulates water and pesticide migrations in both soil micropores and macropores. Its performance is known to be accurate in predicting the fate of pesticides under conventional crop management (Marin-Benito et al., 2014). A commonly cited limitation of MACRO is that the description of the crop is vague and only considers maximum leaf area index, maximum rooting depth, and the maximum crop height (Lammoglia et al., 2017).

Mottes et al., (2014) investigated the effects of agricultural practices on runoff, leaching, erosion, and drift from eight watershed models and nine field models, including the Soil and

Water Assessment Tool (SWAT), Groundwater Loading Effects of Agricultural Management Systems (GLEAMS) and Pesticide Root Zone Model (PRZM). The authors found poor integration of innovative practices. Scaling was also identified as an issue because all models kept the original field-scale representation. Most recently, Queyerel et al., (2016) introduced a simplified pesticide fate module using Simulateur multidisciplinair pour les Cultures Standard or Multidisciplinary Simulator for Standard Crops (STICS) model to better integrate crop management and crop growth in the simulation of agricultural pollution. STICS can simulate growth using a diversity of agricultural practices. Results of STICS were found to be as efficient as most pesticide fate models to predict pesticide leaching (Queyerel et al., 2016). The limitations of STICS are the formalism for water transfer and the absence of non-linear sorption (Queyerel et al., 2016).

For assessing pesticide risks to aquatic organisms, the US EPA Office of Pesticide Programs models pesticide spray drift, runoff and erosion into a hypothetical agricultural pond with specific water body and watershed characteristics using the Pesticide in Water Calculator (PWC). The PWC assumes point source pollution of a contaminant in freshwater ecosystems, but in reality, pollution may be a result of a variety of sources across the landscape (Xie et al., 2018). The PWC simulates daily concentrations for thirty-year exposure distributions for surface water, sediment and interstitial water and its graphic interface can link the output of the PRZM and Variable Volume Water model (VVWM). It assumes maximum use rates and frequencies with the intent to provide estimated concentrations for regulatory purposes (Xie et al., 2018). Represented through a compartmentalized system of water and sediment, the VVWM assumes a complete mass transfer and assumes thermodynamic equilibrium. The VVWM also utilizes abiotic and biotic environmental fate processes on a first order basis. Bulk exchange is

accounted for and is assumed to be described by a first-order mass transfer coefficient. Runoff inflow is predicted by PRZM and assumes that dissolved materials are delivered to the water compartment and sorbed species are delivered to both compartments (Chen et al., 2002). Pesticide inflow through spray drift is comparable to pesticide application rates. PRZM has been used for pesticide regulatory assessments where soil hydrology is relatively homogenous in small-scale and edge-of-field conditions (Chen et al., 2002). The standard farm pond scenario was adapted from Georgia farm ponds and has been recommended by US EPA-OPP to represent a highly vulnerable exposure scenario. This scenario assumes a 10-hectare field treated with pesticides emptying into a 1-hectare x 2 m static farm pond (i.e., fixed water volume with no flow) (Xie et al., 2018). Standardized waterbodies in EPA's models have been widely used in regulatory modelling to represent highly vulnerable exposure scenarios, however, it is not clear whether they represent worst case scenarios or individual regions or states. Perennial and intermittent or ephemeral sloughs, creeks, streams, rivers, agricultural ditches and canals generate significant variability (Xie et al., 2018). The variability in rainfall, topography, vegetative cover, and farming in a watershed complicate scale and accurate predictions of water runoff, soil erosion and off-field loading into water bodies. Predicted concentrations have often been significantly different from monitoring data due to the inability of field models to accurately predict pesticides in waterbodies (Chen et al., 2002). The ability to accurately predict concentrations of agrochemicals is essential for the creation of defensible environmental standards, guidelines and regulations (US EPA, 2017).

The North Carolina Coastal Federation, the U.S. Fish and Wildlife Service's Mattamuskeet National Wildlife Refuge, the North Carolina Wildlife Resources Commission, and a group of community stakeholders have recently developed a Watershed Restoration Plan

for Lake Mattamuskeet (NC Coastal Federation 2018) to assist in the restoration of Submerged Aquatic Vegetation (SAV), water quality, and other aspects of this vital public trust resource. The purpose of this retrospective analysis is to estimate concentrations of targeted herbicides in Lake Mattamuskeet and in its surrounding drainages due to herbicide off-target movement and increases in planted corn and soybean acres since 2004 ($R^2 = 0.59$ in corn and $R^2 = 0.60$ in soybean). Cotton acres have demonstrated a steady decrease ($R^2 = 0.55$) since 2004. In this assessment, we hypothesized that modeled concentrations were less than those associated with adverse effects due to the gathering of information from herbicide labels that were approved for use in North Carolina and that growers followed all label requirements and label restrictions during applications.

MATERIALS AND METHODS

Study Area Description. Lake Mattamuskeet is North Carolina's largest natural lake and is an important stopover for many migratory birds. The lake is 16,720 hectares, 0.61 meters in depth (average), 22.5 kilometers long, and 9.7 kilometers wide (Figure 1) (Moorman et al., 2017).

Approximately 4,430 hectares out of the 11,890 hectares of land draining into Lake Mattamuskeet are intensively farmed, mostly for cotton, soybeans, and corn (Homer et al., 2015). Both PWC assumptions of a 10-hectare field treated with pesticides that empty into a 1-hectare x 2 m static farm pond differ greatly from the actual dimensions of Lake Mattamuskeet.

Lake Mattamuskeet is the centerpiece of the U.S. Fish and Wildlife Service (USFWS) Mattamuskeet National Wildlife Refuge, established in 1934, to maintain and promote wetland habitats for migratory bird populations and specifically wintering waterfowl in and around the lake. Lake Mattamuskeet has incurred SAV declines since 1989 with its eventual extirpation in 2015 from the lake to date (Moorman et al. 2017). There are many potential factors that have

contributed to this situation such as water clarity, water depth, and sediment influx and composition. Among the many threats to SAV, the issue of herbicide exposure and toxic effects is important due to the extensive agriculture production that occurs in the surrounding drainage areas. Herbicides that potentially enter adjacent waterways through agricultural off-target movement can adversely impact nontarget species such as SAV. The lake's native SAV was historically comprised of wild celery (*Vallisneria Americana*), sago pondweed (*Stuckenia pectinata*), southern naiad (*Najas guadalupensis*), redhead grass pondweed (*Potamogeton perfoliatus*) and the branched algae, and muskgrass (*Chara spp.*) and nitella (*Nitella spp.*), among others (Moorman et al., 2017). Lake Mattamuskeet drains a small coastal watershed with little point source or urban influence, which makes Lake Mattamuskeet an ideal setting to study the impacts of agricultural land uses in the coastal plain on shallow lakes (Moorman et al., 2017).

Land Use and Site Selection. The dominant crop types grown in the area surrounding the Lake Mattamuskeet watershed of Hyde County, NC were determined by summarizing yearly USDA crop data from 2004 to 2018 (Figure 2 and Figure 3) (USDA CropScape National Agricultural Statistics Service). Those crops were corn, cotton, soybeans and wheat. A list of herbicides (Table 1) commonly used on those crops was generated with assistance from agricultural and pesticide experts at NC State University and from Hyde County Cooperative Extension personnel. Based on the commonly used herbicide list and literature searches of previous herbicide studies, 31 herbicides were selected for modeling with the PWC.

Pesticide in Water Calculator. The US EPA's Pesticide in Water Calculator v. 1.52 (PWC, <https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/pesticide-water-calculator-version-150-and-152-user>) was utilized to calculate estimated environmental concentrations

(EEC). The PWC links the output from PRZM and VVWM and assumes a fixed volume, no flow, 1 ha by 2 m farm pond or a 5.26 ha by 2.74 m reservoir based on runoff and overspray from an adjacent 10 ha pesticide treated field (Rumschlag et al., 2019). Several parameters are needed to provide estimates for off-target movement including herbicide physical-chemical data, environmental fate data, application method and timing, crop land, and watershed information (EPA Pesticide Chemical Search, International Union of Pure and Applied Chemistry, University of Hertfordshire Pesticide Properties Database, California Department of Pesticide Regulation). The 2018 North Carolina Agricultural Chemical Manual was utilized for application type and application rates (North Carolina Agricultural Chemical Manual, 2018). Product labels were also used to determine targeted crop applications. All information gathered for PWC is captured in Table 1. For all herbicides, water, benthic, and soil reference temperatures were assumed to be 23 degrees C and photolysis reference latitude was 40 degrees. Foliar half-life was only available in literature for sethoxydim, 2,4-D, atrazine, dicamba, metolachlor, flumioxazin, glufosinate, glyphosate, mesosulfuron, mesotrione, and simazine. Per PWC instructions, herbicides without a foliar half-life were left blank. Prepopulated PWC scenarios are developed based on general land cover classes and represent potential pesticide use areas. For this exercise, North Carolina crop scenarios were utilized as available for corn and cotton. In the absence of North Carolina crop scenarios, North Dakota was used for soybeans and wheat. Per the PWC user manual, efficiency was set to 0.99 and drift was 0.01 for all herbicide applications. Meteorological data were downloaded from the US EPA Environmental Modeling Community of Practice for Wilmington, NC due to its nearest proximity to Lake Mattamuskeet. To align with anticipated future field sampling of measured herbicide concentrations at Lake Mattamuskeet, an approximate forty-day average was calculated.

RESULTS AND DISCUSSION

Off-target movement estimates were calculated using herbicide physical-chemical data, environmental fate data, application method and timing and watershed information. Estimated pond concentrations for each targeted herbicide are presented in Table 2. Due to differences in application rate, method, and timing, concentrations are represented by dominant crop type. Overall, 40-day pond concentrations ranged from 0.07 $\mu\text{g/L}$ to 301.5 $\mu\text{g/L}$ (Table 2). For cotton applications, estimated 40-day concentrations ranged from 0.07 $\mu\text{g/L}$ to 301.5 $\mu\text{g/L}$. In soybeans, modelled 40-day pond concentrations ranged from 0.10 $\mu\text{g/L}$ to 149.0 $\mu\text{g/L}$. Herbicides applied to wheat contributed to a minimum of 0.1 $\mu\text{g/L}$ with a maximum of 168.5 $\mu\text{g/L}$. Lastly, corn concentrations ranged from 0.07 $\mu\text{g/L}$ to 96.0 $\mu\text{g/L}$.

Applications of targeted herbicides to cotton generated the highest concentrations with prometryn and alachlor accounting for 301.5 and 229.5 $\mu\text{g/L}$, respectively in the forty-day pond scenario. This can be explained by higher K_{oc} values of 448 and 2318 mL/g for prometryn and alachlor. The organic carbon-water partition co-efficient (K_{oc}) aids in describing how much a chemical substance is adsorbed to soil and is heavily influenced by the organic content (Rutherford et al., 1992). Neither prometryn nor alachlor degrade in the aquatic environment under aerobic or anaerobic conditions (Prometryn and Alachlor - EPA Pesticide Chemical Search). The soil half-life of prometryn was 270 days and 365 days for alachlor (Alachlor - EPA Pesticide Chemical Search). Wheat also contained high estimated concentrations of bentazone and alachlor. Bentazone had a K_{oc} of 34 mL/g and was also stable under aquatic conditions (aerobic and anaerobic) (Bentazone - EPA Pesticide Chemical Search). Bentazone degrades under aqueous photolysis in 3 days (Bentazone - EPA Pesticide Chemical SearchSchematics

demonstrating the potential amount of runoff, drift and erosion into the small pond are illustrated in Figure 4.

Based on USDA crop type acres for 2018, corn and soybeans individually represented almost 40% of acres planted in Hyde County, North Carolina increasing plausibility for concern of the herbicides used on those crops to SAV. In corn, atrazine and metolachlor have the greatest forty-day pond concentrations whereas soybeans have greater forty-day pond concentrations with pyriithiobac. Cotton and wheat represented the least number of planted acres planted in Hyde County, North Carolina, thus creating less concern for prometryn, bentazone and alachlor (labeled for usage in wheat and cotton) and their potential adverse effects on SAV restoration. Comparisons of modeled forty-day pond concentrations with US EPA OPP Aquatic Life Benchmark concentrations for aquatic plants indicate that atrazine, mesosulfuron, simazine, trifloxysulfuron, cloransulam, pyriithiobac, cyanazine, prometryn and alachlor pose the greatest threat to SAV viability. Due to limited labeled crop applications, mesotrione is estimated to have the lowest concentration in pond surface water. Mesotrione has a short aquatic half-life (<4 days) with a soil half-life of 13.5 days (Mesotrione - EPA Pesticide Chemical Search).

Several studies have investigated the effectiveness of surface water models to predict the effect of runoff and spray drift to nearby water bodies. Chen et al. (2002) investigated thirteen pesticides in six Lake Erie tributaries from 1983 to 1991 using the surface water mobility index (SWMI). The SWMI utilizes degradation half-lives and organic carbon-normalized soil/water sorption coefficients to benchmark pesticide mobility relevant to surface water runoff and soil erosion (Chen et al., 2002). These authors found atrazine, simazine and metolachlor within the SWMI range of 0.5 and 0.7 (Chen et al., 2002). By definition, SWMI determines the relative

pesticide mobility scale with 0 being the least mobile and 1 being the most mobile, assuming mobility through surface water runoff and soil erosion (Chen et al., 2002).

Xie et al. (2018) utilized the PWC to generate EECs in California to evaluate the suitability of using US EPA water bodies for the regulatory screening-level exposure assessment in California's agricultural setting. The parametrization of the standard farm pond (dimension and flow) may not physically match the actual studied water body, however, the authors also concluded that the standard pond in exposure modeling was able to provide conservative but realistic estimates of concentrations for the screening-level regulatory exposure assessment in California's agricultural areas. Several pesticides were evaluated in their study in different crop scenarios and estimated concentrations varied greatly from 0.01 $\mu\text{g/L}$ to 71.1 $\mu\text{g/L}$. Simazine was evaluated in strawberries, grapes, corn and alfalfa and subsequent estimated concentrations of simazine in water was estimated at 23.9 $\mu\text{g/L}$ in corn.

Giannouli and Antonopoulos (2015) utilized MACRO and Pesticide Emission Assessment at Regional and Local scales (PEARL) models to simulate the fate of atrazine in a clayey soil by incorporating field data from an irrigated field cropped with corn in Greece. Both MACRO and PEARL are one-dimensional models that describe water flux, heat, and solute transport in a soil matrix. Both models account for a complete water balance including water flow, canopy interception and root water uptake, seepage to drains and groundwater. According to statistical criteria, both MACRO and PEARL provide a reliable simulation of the mass transport of atrazine, although both models underestimate atrazine concentrations in the subsoil (Giannouli and Antonopoulos 2015). Similarly, the PWC appears to accurately predict herbicides of potential concern.

Zhang and Goh (2015) evaluated PRZM, RZWQM, and OpusCZ models for simulation of pesticide movement generated by irrigation and rainfall to address the data gap in modeling irrigation off-target movement and the regulatory needs in California. The models were evaluated on their accuracy in predicting pesticide movement using measurements from three field studies conducted at agricultural fields in California. Simulated results were compared with measured data on runoff volume, sediment erosion, and pesticide mass in runoff. All three models were capable of predicting pesticides at field edge, however, they differed when comparing hydrological parameters. All three models were noted to simulate water and pesticide movement with good accuracy for events generated by natural rainfall or sprinkler irrigation (Zhang and Goh 2015). Ma et al., (2000) compared the Groundwater Loading Effects of Agricultural Management System (GLEAMS), Opus, PRZM2b, and PRZM3 models using a 2-yr field study with simulated rainfall events. They found that GLEAMS, Opus, PRZM2b, and PRZM3 adequately predicted water off-target movement. All three models were able to simulate the amount of water runoff, sediment erosion, and simazine in off-target movement with good accuracy (Ma et al., 2000). In comparison, our PWC concentrations reflect worst case off-target movement to a small pond adjacent to a ten-hectare farm. The EECs were less than water quality guidelines from Canada and EPA (Table 2).

Hartz et al., (2017) utilized the PWC model to estimate the concentration of in-furrow insecticides applied to maize. The concentrations of tefluthrin and clothianidin were sampled from a field in Illinois and were compared to PWC modelled concentrations in surface runoff. For tefluthrin, the observed environmental concentration (OEC) after application in 2012 and 2013 were two to six times higher than the EEC derived from PWC. Clothianidin OEC and EEC were poorly correlated with EECs being underpredicted. The authors attribute the under-

estimation to an additional route of transport for clothianidin from the seed coating for runoff. The addition of particulate matter at application subsequently improved the agreement of EEC and OEC for clothianidin (Hartz et al., 2017). The authors also noted that the selection of the application method has a direct impact on the estimation of the tefluthrin and clothianidin.

Several regulatory guidelines and standards were utilized to compare the risk of SAV restoration to PECs from PWC. The US EPA OPP Aquatic Life Benchmarks are based on toxicity values from EPA reviewed scientific studies and values are used to estimate risk for pesticides and their degradates in ecological risk assessments written in support of pesticide registration (EPA OPP Aquatic Life Benchmarks). For acute vascular plants, the toxicity value is a short-term (less than 10 days) EC₅₀ study with duckweed (EPA OPP Aquatic Life Benchmarks). The North Carolina In-Stream Target Values for Surface Water were designed to protect both fresh and saltwater aquatic organisms to promote biological integrity. The North Carolina In-Stream Target Values are based on a chronic 96-hour LC₅₀ using either the Dungeness crab (*Metacarcinus magister*), opossum shrimp (*Mysis diluviana*) or the northern pink shrimp (*Pandalus borealis*) (NC Division of Water Resources). Values are calculated by multiplying the LC₅₀ from a chronic 96-hour study by a safety factor of 0.01. Similarly, CCME Water Quality guidelines are intended to provide protection of freshwater and marine life from anthropogenic stressors. Guidelines are numerical limits based available scientifically defensible toxicological data and guideline values meant to protect all forms of aquatic life and all aspects of the aquatic life cycles, including the most sensitive life stage of the most sensitive species over the long term. CCME Water Quality values are also derived from 96-hour chronic studies (CCME Protocol, 2007). Although our PECs encompass a 40-day deployment duration and extend well beyond the 96 hour chronic exposure guidelines for aquatic organisms comparisons

provide the best estimate of risk to aquatic plants. Generated PECs are also limited in predicting the cumulative role of mixtures.

Based on our PWC results for the 31 targeted herbicides, known US EPA OPP Aquatic Life Benchmark standards and current herbicides of concern in the literature, several herbicides may have the potential for inhibiting the restoration of SAV at Lake Mattamuskeet.

Comparisons of modeled forty-day pond concentrations with US EPA OPP Aquatic Life Benchmark concentrations for aquatic plants indicate that atrazine, mesosulfuron, simazine, trifloxysulfuron, cloransulam, pyriithiobac, cyanazine, prometryn and alachlor pose the greatest threat to surface water off-target movement and subsequent SAV exposure (Table 2). However, based on Hyde County total planted acres for 2018, applications of herbicides to corn and soybeans may be of particular concern. In corn, atrazine and metolachlor have the highest forty-day pond concentrations whereas soybeans have higher forty-day pond concentrations with pyriithiobac. In addition to predicted environmental concentrations, Figure 4 illustrates the amount of active ingredient that would run off from agricultural land into simulated EPA small pond. Out of the recommended application rate per each herbicide, the red values highlight the amount that will eventually reach the water body. The main mechanism of transport from the field to the water are depicted near the blue arrow in the form of erosion, spray drift or runoff. Pyriithiobac has the smallest application rate of 0.32 lbs/acre and 4.1% of the active ingredient is expected to reach the water body. Erosion and spray drift account for 1.0% and 2.4% of offsite movement, respectively. Runoff accounts for 96.5% of movement to the small water body.

Potential herbicide distribution into surface water differed per herbicide. Runoff was expected to be the dominant route of movement into surface water for both atrazine (94.2%),

metolachlor (88.1%) and pyriithiobac (96.5%). In general, herbicides with low to moderate K_{oc} values and high water solubility migrated to surface water through runoff.

In conclusion, the environmental fate of agrochemicals is quite complex and depends on multiple chemical and environmental factors such as soil and water chemistry, partition coefficients and abiotic and biotic degradation both in water and in soil (Senesi, 1983). Estimated environmental concentrations from fate and transport models are utilized by regulatory agencies because they provide consistent methodology for estimates across compounds and landscapes. Current models such as the PWC used in this study provide useful estimates when surface water measurements are lacking. In the future, active monitoring of herbicide concentrations in surface water combined with risk analysis could provide growers with management tools to help mitigate the environmental risk of agrochemicals entering aquatic ecosystems, thereby aiding in the recovery of SAV in the APES, and in particular at Lake Mattamuskeet.

Reducing the risks of pesticide exposure in surface waters is a common goal in retrospective risk assessments. Based on the current literature, ecological scenarios for ditches, streams, and ponds are key components that are missing in surface water evaluations. A frequently discussed limitation of the PWC in the literature is that a single point source of contamination is assumed. Complex mixtures of chemicals like those encountered in surface waters are thus not estimated. We suggest that active pesticide monitoring combined with field-level experiments should be performed in combination with modeling exercises to provide insight into which concentrations might be detrimental and which agricultural practices might be the most effective at reducing off-target movement and exposure.

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TABLES

Table 1. A list of 31 targeted herbicides were derived for modeling and subsequent environmental fate data based on their use on the dominant crop types (corn, cotton, soybeans, wheat) grown in Hyde County, North Carolina. Data were derived from EPA's Online Pesticide Search.

	2,4-D	Atrazine	Dicamba	Flumioxazin	Fomesafen	Glufosinate
K_{OC}/K_D (mL/g)	0.58	75	1.42	557	60	430
Water Column Metabolism Half-Life (day)	15	155	26	4	7	24.5
Water Reference Temperature (degrees C)	22	22	22	22	22	22
Benthic Metabolism Half-life (day)	312	608	20	0.2	7	37
Aqueous Photolysis Half-life (day)	13	0.2	12.3	5.6	stable	stable
Hydrolysis Half-life (day)	39	stable	stable	1	stable	stable
Soil Half-life (day)	66	146	31	14.7	288	16
Soil Reference Temperature (degrees C)	22	22	22	22	22	22
Foliar Half-life (day)	9	5	9	10	stable	35
Molecular Weight (g/mol)	221.04	215.7	221.04	354	438.77	198.2
Vapor Pressure (torr)	1.4E-07	3.00E-07	1.00E-07	2.40E-06	7.5E-07	2.32E-07
Solubility (mg/L)	33900	33	4500	1.8	6.00E+05	1.37E+06

Table 1. (continued)

	Glyphosate	Mesosulfuron	Mesotrione	Metolachlor	Simazine	Trifloxysulfuron
K_{OC}/K_D (mL/g)	24000	48	80.5	200	123	306
Water Column Metabolism Half-Life (day)	7	57	5	39	213	21.5
Water Reference Temperature (degrees C)	22	22	22	22	22	22
Benthic Metabolism Half-life (day)	8.1	6.74	4	78.2	168	stable
Aqueous Photolysis Half-life (day)	stable	stable	86.8	stable	stable	21.4
Hydrolysis Half-life (day)	stable	stable	stable	stable	stable	41.1
Soil Half-life (day)	96.4	41.5	13.5	26	110	63.5
Soil Reference Temperature (degrees C)	22	22	22	22	22	22
Foliar Half-life (day)	3	35	3.9	20	5	35
Molecular Weight (g/mol)	169.07	503.5	339.32	283.46	201.66	459.33
Vapor Pressure (torr)	1.84E-07	2.63E-14	4.30E-08	3.10E-05	6.1E-09	1.00E-07
Solubility (mg/L)	11600	480	160	530	6.2	25700

Table 1. (continued)

	Acetochlor	Carfentrazone	Cloransulam	Flumiclorac	Fluometuron	MSMA
K_{OC}/K_D (mL/g)	139	750	915	30	98.7	2850
Water Column Metabolism Half-Life (day)	26.6	3.5	25.6	stable	stable	245
Water Reference Temperature (degrees C)	22	22	22	22	22	22
Benthic Metabolism Half-life (day)	251	0.8	16	stable	0.2	stable
Aqueous Photolysis Half-life (day)	0	8	1	0.8	1	stable
Hydrolysis Half-life (day)	0	8.6	231	0.8	19	stable
Soil Half-life (day)	13.3	1.3	13	2.5	17.5	180
Soil Reference Temperature (degrees C)	22	22	22	22	22	22
Foliar Half-life (day)	stable	stable	stable	stable	stable	stable
Molecular Weight (g/mol)	269.77	412.19	429.82	423.86	423.9	162.0
Vapor Pressure (torr)	2.80E-05	1.20E-07	3.00E-16	1.00E-05	5.00E-07	9.96E-06
Solubility (mg/L)	233	12000	184000	0.89	111	1040000

Table 1. (continued)

	Pinoxaden	Pyriithiobac	Pyroxsulam	Thifensulfuron	Tribenuron	Bentazone
K_{oc}/K_D (mL/g)	403	0.5	0.60	28.3	35	34
Water Column Metabolism Half-Life (day)	stable	stable	128	22.8	stable	stable
Water Reference Temperature (degrees C)	22	22	22	22	22	22
Benthic Metabolism Half-life (day)	1	stable	stable	stable	11	stable
Aqueous Photolysis Half-life (day)	stable	13	3.2	94	stable	3
Hydrolysis Half-life (day)	10	stable	stable	180	6	stable
Soil Half-life (day)	3	60	3.3	1.39	14	20
Soil Reference Temperature (degrees C)	22	22	22	22	22	22
Foliar Half-life (day)	stable	stable	stable	stable	stable	stable
Molecular Weight (g/mol)	400.5	348.74	434.35	373.0	395.4	240.3
Vapor Pressure (torr)	3.43E-09	3.6E-08	1.00E-09	3.89E-08	3.80E-10	3.67E-06
Solubility (mg/L)	200	728000	3200	54.1	2483	570

Table 1. (continued)

	Diclofop	Paraquat	Sethoxydim	Cyanazine	Prometryn	Alachlor
K_{OC}/K_D (mL/g)	4.89	40000000	100	190	448	2318
Water Column Metabolism Half-Life (day)	0.06	stable	stable	84	stable	500
Water Reference Temperature (degrees C)	22	22	22	22	22	22
Benthic Metabolism Half-life (day)	stable	stable	stable	stable	stable	stable
Aqueous Photolysis Half-life (day)	31.7	stable	5	stable	stable	stable
Hydrolysis Half-life (day)	stable	stable	155.2	stable	stable	365
Soil Half-life (day)	1	stable	5	116	270	30
Soil Reference Temperature (degrees C)	22	22	22	22	22	22
Foliar Half-life (day)	stable	stable	3	stable	stable	stable
Molecular Weight (g/mol)	341.19	257.2	327.50	240.69	241.37	269.77
Vapor Pressure (torr)	1.87E-04	1.00E-07	4.55E-10	2.13E-05	1.0E-06	2.20E-05
Solubility (mg/L)	50	620000	25	171	33	170

Table 1. (continued)

	Nicosulfuron	Dimethipin
K_{OC}/K_D (mL/g)	78.8	42
Water Column Metabolism Half-Life (day)	63	470
Water Reference Temperature (degrees C)	22	22
Benthic Metabolism Half-life (day)	stable	stable
Aqueous Photolysis Half-life (day)	stable	224
Hydrolysis Half-life (day)	15	stable
Soil Half-life (day)	26	183
Soil Reference Temperature (degrees C)	22	22
Foliar Half-life (day)	stable	stable
Molecular Weight (g/mol)	410.4	210.27
Vapor Pressure (torr)	1.20E-16	3.81E-07
Solubility (mg/L)	1200	4600

Table 2. Estimated forty-day pond concentrations were calculated using 31 herbicides used on dominant crop types grown in the Lake Mattamuskeet watershed of Hyde County, North Carolina utilizing the Pesticide in Water Calculator and environmental fate data obtained from the published literature and herbicide labels. Models were run under an assumed 10 hectare adjacent field that drains into a hypothetical 1 hectare by 1 m deep pond.

Compound	US EPA OPP Aquatic Life Benchmark for Vascular Plants ($\mu\text{g/L}$)	Estimated 40 Day Pond Concentrations ($\mu\text{g/L}$)			
		Corn	Cotton	Soybean	Wheat
2,4-D	299.2	30.0	x	x	14.7
Atrazine	4.6	63.8	x	x	x
Dicamba	>3250	36.6	47.0	32.7	46.1
Flumioxazin	0.49	0.1	0.1	0.1	0.1
Fomesafen	210	x	8.7	5.1	x
Glufosinate	1470	20.9	34.6	26.0	x
Glyphosate	11900	1.4	1.5	0.9	x
Mesosulfuron	0.64	x	x	x	0.23
Mesotrione	17.7	1.2	x	x	x
Metolachlor	21	19.9	13.0	9.0	x
Simazine	67	96.0	x	x	x
Trifloxysulfuron	0.24	x	0.50	x	x
Carfentrazone	5.9	0.08	0.12	0.16	0.12
Cloransulam	0.99	x	x	x	0.12
Flumiclorac	>35	x	0.27	x	0.38
Fluometuron	220	x	x	1.17	x
MSMA	104000	x	149	x	x
Pinoxaden	2900	2.3	x	x	x
Pyriithiobac	n/a	x	x	42.4	x
Pyroxsulam	2.57	0.13	0.07	0.14	0.26
Thifensulfuron	1.59	0.35	0.22	x	0.36
Tribenuron	2	0.07	x	x	x
Bentazone	5350	x	46.5	x	159.5
Diclofop	1295	0.08	x	x	x
Paraquat	71	3.7	5.8	6	x
Sethoxydim	210	x	4.6	7.2	6.6
Cyanazine	n/a	x	46.4	x	x
Prometryn	11.9	x	301.5	x	x
Alachlor	2.3	44.3	x	34.8	x
Nicosulfuron	n/a	x	0.4	x	x
Dimethipin	2100	x	x	39.7	x

<https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/pesticide-water-calculator-version-150-and-152-user>; n/a Not available; EPA Environmental Protection Agency

Table 2. (continued)

Compound	CCME Water Quality Guideline for Protection of Aquatic Life, Freshwater ($\mu\text{g/L}$)	Estimated 40 Day Pond Concentrations ($\mu\text{g/L}$)			
		Corn	Cotton	Soybean	Wheat
2,4-D	4	30.0	x	x	14.7
Atrazine	1.8	63.8	x	x	x
Dicamba	10	36.6	47.0	32.7	46.1
Flumioxazin	n/a	0.1	0.1	0.1	0.1
Fomesafen	n/a	x	8.7	5.1	x
Glufosinate	n/a	20.9	34.6	26.0	x
Glyphosate	27,000	1.4	1.5	0.9	x
Mesosulfuron	n/a	x	x	x	0.23
Mesotrione	n/a	1.2	x	x	x
Metolachlor	7.8	19.9	13.0	9.0	x
Simazine	10	96.0	x	x	x
Trifloxysulfuron	n/a	x	0.50	x	x
Carfentrazone	n/a	0.08	0.12	0.16	0.12
Cloransulam	n/a	x	x	x	0.12
Flumiclorac	n/a	x	0.27	x	0.38
Fluometuron	n/a	x	x	1.17	x
MSMA	n/a	x	149	x	x
Pinoxaden	n/a	2.3	x	x	x
Pyrithiobac	n/a	x	x	42.4	x
Pyroxsulam	n/a	0.13	0.07	0.14	0.26
Thifensulfuron	n/a	0.35	0.22	x	0.36
Tribenuron	n/a	0.07	x	x	x
Bentazone	n/a	x	46.5	x	159.5
Diclofop	6.1	0.08	x	x	x
Paraquat	n/a	3.7	5.8	6	x
Sethoxydim	n/a	x	4.6	7.2	6.6
Cyanazine	2	x	46.4	x	x
Prometryn	n/a	x	301.5	x	x
Alachlor	n/a	44.3	x	34.8	x
Nicosulfuron	n/a	x	0.4	x	x
Dimethipin	n/a	x	x	39.7	x

<https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/pesticide-water-calculator-version-150-and-152-user>; n/a Not available; CCME Canadian Council of Ministers of the Environment

Table 2. (continued)

Compound	NC In-Stream Target Values for Surface Waters ($\mu\text{g/L}$)	Estimated 40 Day Pond Concentrations ($\mu\text{g/L}$)			
		Corn	Cotton	Soybean	Wheat
2,4-D	60	30.0	x	x	14.7
Atrazine	640	63.8	x	x	x
Dicamba	200	36.6	47.0	32.7	46.1
Flumioxazin	n/a	0.1	0.1	0.1	0.1
Fomesafen	n/a	x	8.7	5.1	x
Glufosinate	n/a	20.9	34.6	26.0	x
Glyphosate	n/a	1.4	1.5	0.9	x
Mesosulfuron	n/a	x	x	x	0.23
Mesotrione	n/a	1.2	x	x	x
Metolachlor	240	19.9	13.0	9.0	x
Simazine	n/a	96.0	x	x	x
Trifloxysulfuron	n/a	x	0.50	x	x
Carfentrazone	n/a	0.08	0.12	0.16	0.12
Cloransulam	n/a	x	x	x	0.12
Flumiclorac	n/a	x	0.27	x	0.38
Fluometuron	n/a	x	x	1.17	x
MSMA	n/a	x	149	x	x
Pinoxaden	n/a	2.3	x	x	x
Pyrithiobac	n/a	x	x	42.4	x
Pyroxsulam	n/a	0.13	0.07	0.14	0.26
Thifensulfuron	n/a	0.35	0.22	x	0.36
Tribenuron	n/a	0.07	x	x	x
Bentazone	n/a	x	46.5	x	159.5
Diclofop	n/a	0.08	x	x	x
Paraquat	n/a	3.7	5.8	6	x
Sethoxydim	n/a	x	4.6	7.2	6.6
Cyanazine	n/a	x	46.4	x	x
Prometryn	n/a	x	301.5	x	x
Alachlor	n/a	44.3	x	34.8	x
Nicosulfuron	n/a	x	0.4	x	x
Dimethipin	n/a	x	x	39.7	x

<https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/pesticide-water-calculator-version-150-and-152-user>; n/a Not available; NC North Carolina

FIGURES

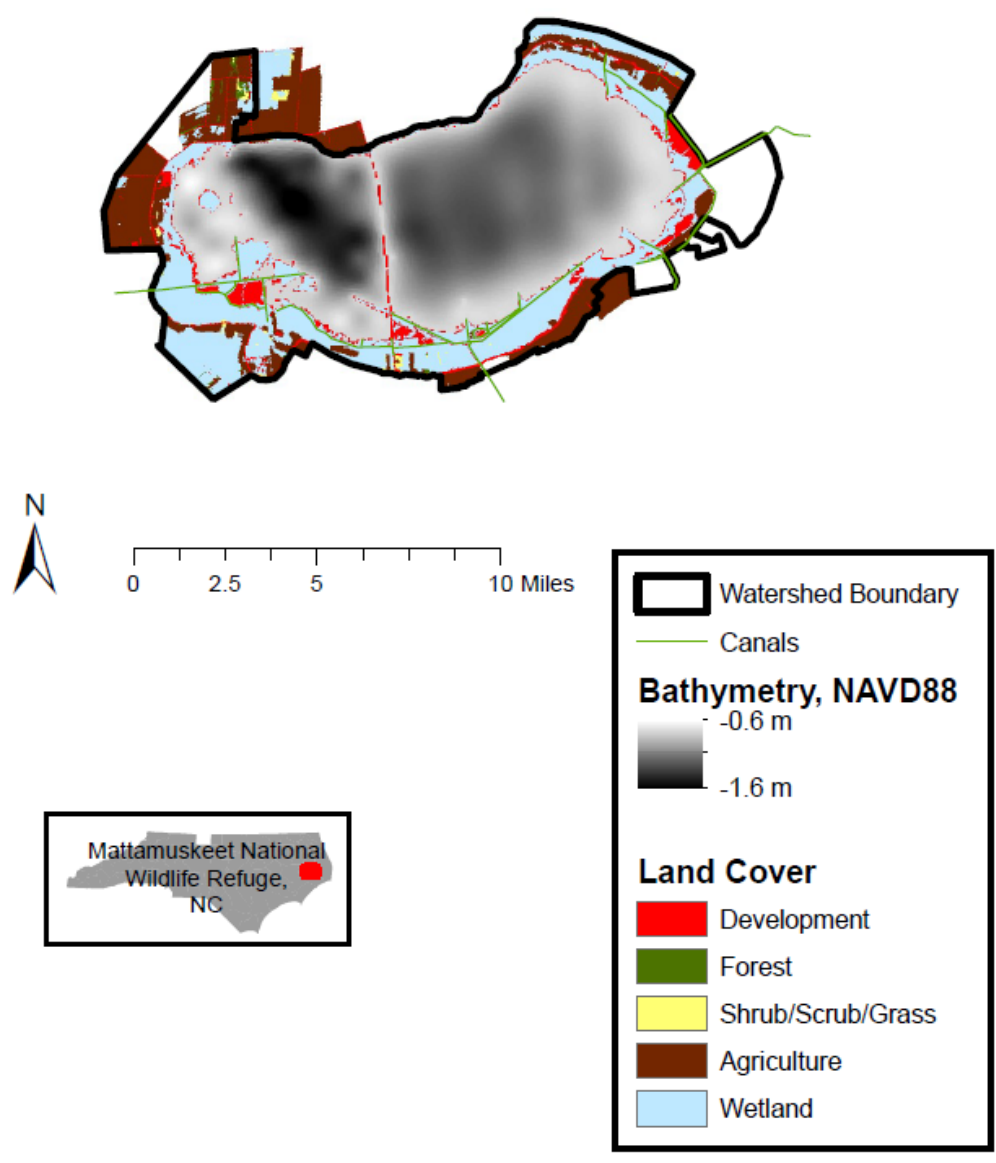


Figure 1. A map of Lake Mattamuskeet located in Eastern North Carolina adapted from Moorman et al., 2017.

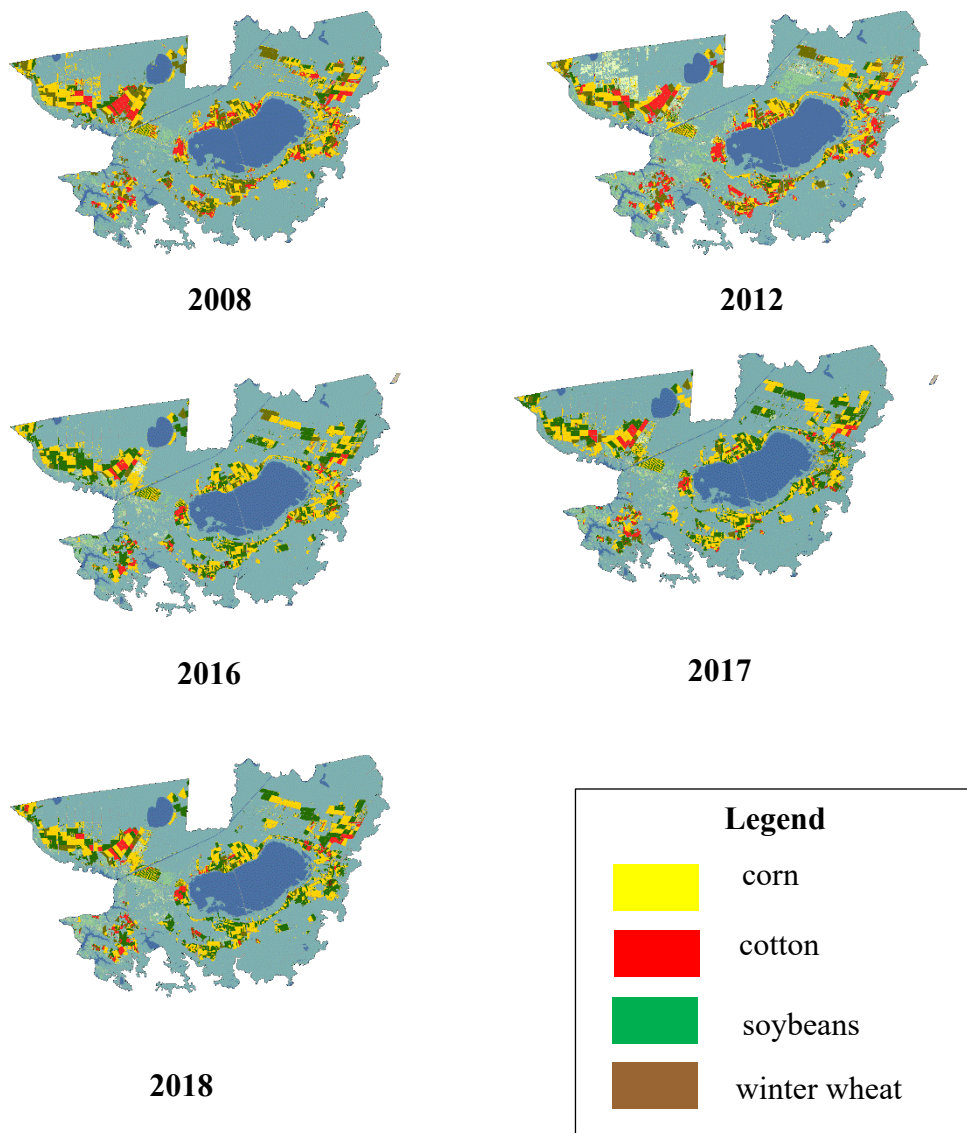


Figure 2. Crop Land Layers from USDA CropScape indicate agricultural shifts in Hyde County, North Carolina from 2008 through 2018

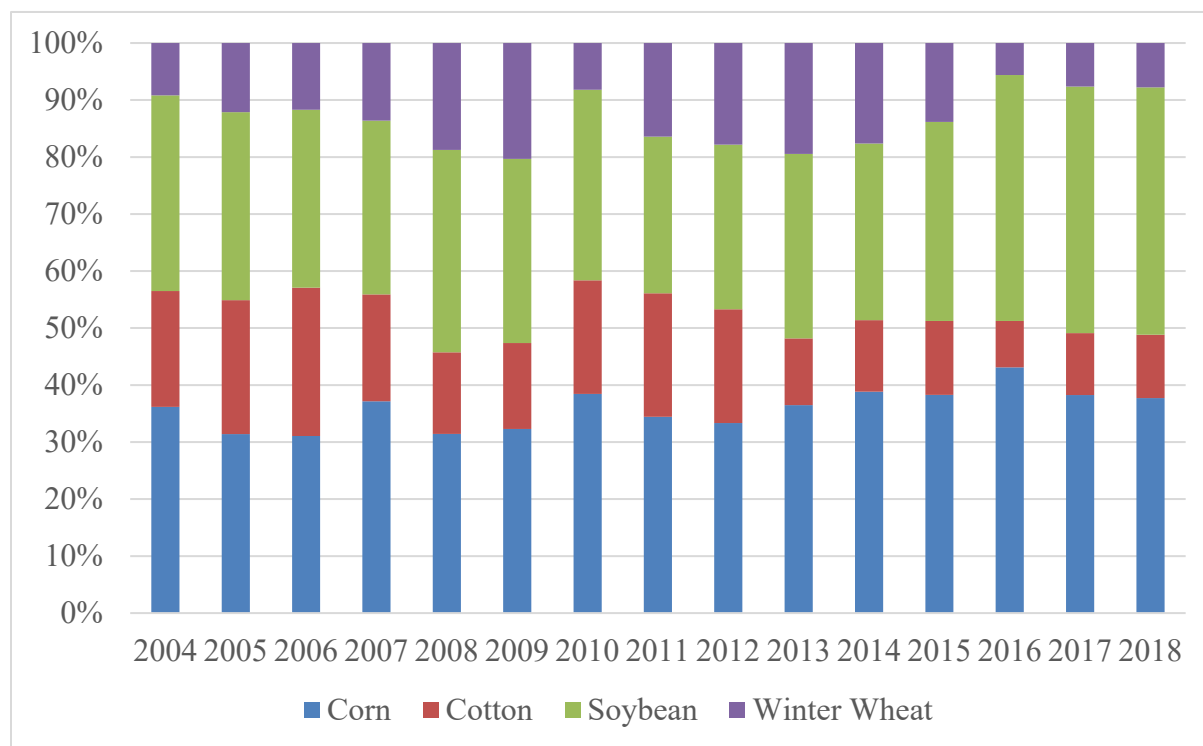
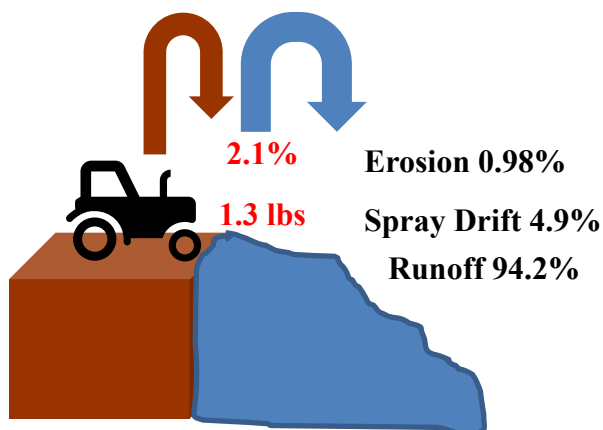


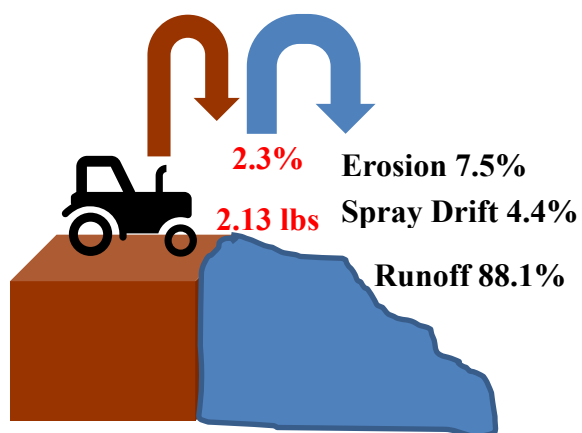
Figure 3. Dominant crop distributions from 2004 through 2018 demonstrate shifts in crop type in Hyde County, North Carolina. A linear regression for each crop type over time demonstrated an increase in planted corn and soybean acres ($R^2 = 0.59$ in corn and $R^2 = 0.60$). A decrease in cotton acres was demonstrated since 2004 ($R^2 = 0.55$). Data were analyzed from USDA CropScape.

Atrazine Model

Maximum application rate:
2.5 lbs/acre

**Metolachlor Model**

Maximum application rate:
3.75 lbs/acre

**Pyriithiobac Model**

Maximum application rate:
0.32 lbs/acre

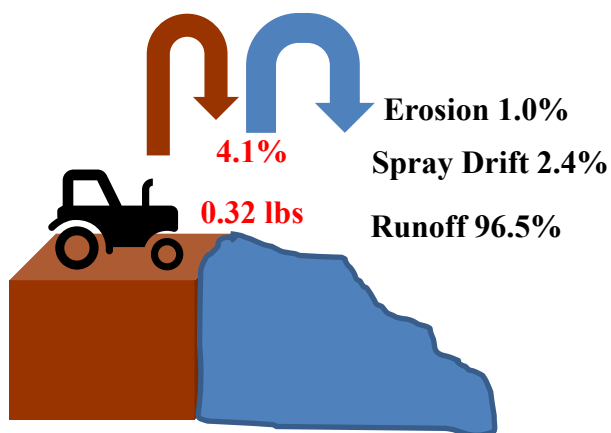


Figure 4. A surface water distribution schematic demonstrating the amount of runoff, drift and erosion after application of atrazine, metolachlor, pyriithiobac.. Calculations were based on 10 hectare (24.7 acre) adjacent field.

**CHAPTER 3. Assessing Agricultural Herbicide Off-target Movement in a Coastal Plain
Watershed with Polar Organic Integrative Samplers (POCIS)**

ABSTRACT

Off-target movement of herbicides from agricultural applications into surface waters have the potential to negatively influence aquatic biota such as submerged aquatic vegetation (SAV). Herbicide exposure and toxicity to SAV is dependent upon many factors such as the rate of herbicide accumulation and the ability of the plant to recover after exposure. We measured concentrations of 12 targeted herbicides at five locations in the Lake Mattamuskeet, North Carolina watershed using four sequential sets of Polar Organic Chemical Integrative Samplers (POCIS) deployed at approximately six-week intervals during the active crop planting and growing season in Eastern North Carolina in 2018. Among all POCIS deployment periods, atrazine and metolachlor were the most common herbicide detected at Lake Mattamuskeet, with concentrations of atrazine ranging from 0.0 – 0.277.5 $\mu\text{g/L}$. The highest concentrations of atrazine and metolachlor were found between May and July of 2018 when concentrations of metolachlor ranged from 0 – 0.15 $\mu\text{g/L}$. We also detected concentrations of 2,4 -D and simazine during the May – July POCIS deployment. The simazine time weighted average concentrations were relatively low, ranging from 0 – 0.002 $\mu\text{g/L}$ and 2,4-D concentrations ranged from 0 – 0.122 $\mu\text{g/L}$. Other chemicals such as DEET, azoxyxystrobin, methoxyfenozide, atratone, and metolachlor morpholinone were detected during a nontargeted analysis and emphasize that other compounds present in the water could also pose a potential threat to restoration and recovery of SAV at Lake Mattamuskeet.

INTRODUCTION

The off-target movement of herbicides from agricultural applications into surface waters have the potential to negatively influence aquatic biota such as submerged aquatic vegetation (SAV). Identifying input sources and magnitudes are needed to better define impactful reduction and best management practices. Lake Mattamuskeet is North Carolina's largest natural lake and a prominent feature on the Albemarle-Pamlico peninsula. With the exception of Florida, North Carolina has the most extensive acreage of SAV of any state of the Atlantic coast with approximately 99% of SAV found in the Albemarle-Pamlico Estuary (Powell et al., 2017). The SAV in Lake Mattamuskeet has declined since 1989 and ongoing poor water quality, including agricultural herbicide influx, may hamper its restoration and recovery (Moorman et al., 2017). It has been presumed that the SAV declines have been due to the conversion of croplands that drain into the lake. Shifts in agricultural land use may impact water quality and subsequent SAV production and health. The United States Department of Agriculture (USDA) CropScape data was further analyzed and an approximate increase of 600 planted acres per year of corn and an increase of approximately 700 acres per year of soybeans ($R^2 = 0.59$ for corn and $R^2 = 0.60$ soybeans). Cotton acres decreased by approximately 700 acres per year ($R^2 = 0.55$) while winter wheat remained constant ($R^2 = 0.01$). These results demonstrate the potential to introduce new contaminants of concern (Chapter 2). The impact of these contaminants could significantly degenerate SAV production while having a profound effect on water quality and its subsequent ecosystem services.

Since 1991, fluctuations in chlorophyll α , total nitrogen, total phosphorus, total suspended solids, turbidity, and pH measurements strongly suggest the lake has become eutrophic. Many water quality parameters have become elevated above published guidelines and

standards since 2012 (Moorman et al., 2017). Sixty-eight percent of the chlorophyll α samples collected during 2012–2015 were above the North Carolina (NCOAH 2016) regulatory water-quality standard of 40 $\mu\text{g/L}$ (56 of 82 samples) (Moorman et al., 2017). These authors also suggested that the increase in total suspended sediments and turbidity have been accentuated by the loss of SAV. Increasing concentrations of nutrients and phytoplankton are all primary factors limiting SAV growth and survival in the lake and have been associated with proliferation of cyanobacterial blooms in Lake Mattamuskeet (Moorman et al., 2017).

Herbicide exposure and toxicity to SAV are dependent upon many factors such as the rate of herbicide uptake and the ability of the plant to recover after exposure (Cedergreen et al., 2005). Toxicokinetic models for uptake and metabolism in higher aquatic plants currently do not exist (Cedergreen et al., 2005). The rate of herbicide uptake and accumulation is largely dependent upon the physical and chemical properties of the compound, the rate of uptake, the rate of inactivation and the rate of herbicide release (Cedergreen et al., 2005). Transport of agrochemicals is often facilitated by surface and subsurface drainage systems mainly in drainage canals and tile drains that lead into larger bodies of water such as lakes or streams (Dalton et al., 2015). Identifying point sources and subsequent characterization of agrochemicals into the aquatic ecosystem can be dynamic, problematic and complex.

Therefore, in-situ sampling is required to capture agrochemical inputs into impacted surface waters. Grab or spot samples are commonly used to characterize pesticide residues in surface waters due to the simplicity and ease of use but often lack temporal representation (Poulier et al., 2014). To overcome the lack of temporal representation, novel passive sampling devices were developed for deployment over a period of time to accumulate contaminants; first was the semi-permeable membrane device for organic compounds with a $\log K_{ow} \leq 4$ (Huckins

et al., 2002; Alvarez 1999; Alvarez et al., 2004). Subsequently, polar organic chemical integrative samplers (POCIS) were developed for use with compounds with a $0 \leq \log K_{ow} \leq 5$ (Alvarez 1999; Alvarez et al., 2004). With these passive samplers, contaminants are continuously accumulated and are subsequently concentrated to allow lower quantification limits (LOQ) (Guibal et al., 2018). The use of POCIS samplers has been successfully applied in several environmental media such as surface waters and sewage effluents (Gonzalez-Rey et al., 2015). A time weighted average (TWA) can be calculated to provide an overall estimate of contaminant concentrations. In contrast to grab or spot sampling, if the period of deployment is too short, contaminant concentrations can have a TWA lower than LOQ (Guibal et al., 2018). A drawback associated with utilizing POCIS samplers are the necessity of calculating accurate concentrations using the target compound sampling rate (R_s) (L/day) (Dalton et al., 2014; Bueno et al., 2009). For each analyte, the R_s must be determined through laboratory experiments using flow through or static renewal microcosm exposure systems and depend on its inherent physical chemical properties and environmental conditions (Roberts and Bersuder, 2006).

The goal of this study was to measure concentrations of targeted herbicides at five locations in or around the watershed that drains into Lake Mattamuskeet using POCIS samplers during the active crop planting and growing season in Eastern North Carolina in 2018. The specific objectives were to use POCIS for measuring TWA concentrations of herbicides at Lake Mattamuskeet based on the crop type and distribution and dominant herbicides used on each in the agricultural lands surrounding the lake and to compare the measured concentrations to those from modeled off-target scenarios. The methodology used herein may be adapted to other locations for improved land and herbicide management goals.

MATERIALS AND METHODS

In-Situ POCIS Sampling Locations. A total of five locations were selected for POCIS sampling for targeted herbicides in this study (Figure 5); four were in agricultural drainage canals leading directly to Lake Mattamuskeet and one was in the Lake within the western basin at a permanent U.S. Geological Survey (USGS) monitoring station. The five locations were chosen based on optimal spatial land use coverage of the watershed and the main potential influx points for herbicides into the lake. The five sites were identified as north canal, south canal, east canal, west canal, and west in-lake (Table 3). Ground truthing was performed during the 2018 growing season and confirmed the prevalence of corn and cotton in the East and West sampling locations. Soybeans and corn were dominant crop types at the North and South sampling sites. Ground truthing crop types are consistent with USDA CropScape estimates for 2018 (see Chapter 2).

Narrowed Targeted Herbicides and In-Situ POCIS Sampling Intervals. A list of 31 herbicides most commonly applied to the dominant crops (corn, cotton, soybean, and wheat) grown in the watershed surrounding Lake Mattamuskeet was generated by crop and pesticide experts with NC State University and Hyde County Cooperative Extension. Predicted environmental concentrations of these 31 herbicides were modeled (see Chapter 2) to determine those posing the greatest potential risk to SAV. From the modeling exercise and subsequent assessment, a list of 12 target commonly used herbicides was derived for further investigation with POCIS sampling and analysis in this study (Table 4). Concentrations of 12 targeted herbicides were sampled at the five locations in the Lake Mattamuskeet, North Carolina watershed using four sequential sets of POCIS deployed at approximately six-week intervals during the active crop planting and growing season in Eastern North Carolina in 2018 (Table 3).

The four sampling events took place from April 11 – May 24 (POCIS set 1), May 24 – July 6 (POCIS set 2), July 6 – August 16 (POCIS set 3), and August 16– September 28 (POCIS set 4) and spanned the largest application periods for the major crops grown in the watershed. Three of the sets of POCIS (sets 1, 3, and 4) were purchased and analyzed by NC State University whereas set 2 was purchased and analyzed by the USGS as an added comparative and validation piece. POCIS sets 1, 3 and 4 and POCIS set 2 were chemically analyzed by different laboratories.

POCIS

POCIS Deployment, Retrieval, and Analysis. The POCIS used in deployments 1, 3, and 4 were purchased from Environmental Sampling Technologies, Inc. (EST) in St. Joseph, MO. The POCIS technology is well documented in the published literature for measuring a variety of aquatic contaminants (Van Metre et al., 2017; Dalton et al., 2014; Mhadhbi et al., 2019) and is covered (manufacture and assembly) under U.S. Government patent number 6,478,961 B2). Briefly, the POCIS were composed of two sheets of microporous (0.1 μ m pore size) polyethersulfone membrane encasing a solid phase sorbent, Oasis HLB (see Figure 6 for visual representation). The Oasis HLB is a universal solid-phase extraction sorbent widely used for sampling a large range of hydrophilic to lipophilic organic chemicals from water. Three replicate POCIS disks were attached to a stainless-steel holder to help facilitate improved sampling and quantification of targeted herbicides (<http://www.est-lab.com/>). For deployment at our sites, the stainless-steel holder was then placed into a paint canister provided by EST with holes drilled throughout the sides, top, and bottom to allow for adequate flow of water and to protect the POCIS from environmental debris. The POCIS were then deployed at a site within the paint canister by a rope attached to a stable structure on shore and also tied to a brick to

prevent movement off location. Several small floats were affixed to the top of the paint can to ensure that the POCIS was in the water column and to prevent sediment fouling. A schematic of the POCIS deployment configuration is shown in Figure 7. Field blanks were utilized to ensure data quality and contained a set of 3 POCIS in a stainless-steel holder within a paint can (without holes). During each site visit, a field blank was opened at a randomly selected sampling location. The sample was then closed back into the stainless-steel paint canister after all sampling work was complete.

After field retrieval of POCIS samplers, the POCIS disks were removed from the canister and placed in a food grade sealable plastic bag and immediately placed in a cooler with ice and transported to the laboratory (including field blank). Once at the laboratory, the POCIS were stored in the freezer at -20 °C until shipment to the laboratory where they were stored frozen until extraction and further laboratory processing.

POCIS sets 1, 3 and 4 were shipped back to EST Inc. and extracted according to their established protocol (EST Laboratories, SOP E-52). The POCIS film was removed from the apparatus in triplicate, rinsed with methanol and then extracted using OASIS HLB with 25 mL of methanol. Each extract from the same site and deployment date were then pooled to form one composite sample and concentrated to a final volume of approximately 5 mL in methanol. Sample extracts were shipped back to NC State University and at the time of analysis extract volumes were measured and approximately 10% of each sample was removed, transferred to a labeled tube and then taken to dryness using a SpeedVac system. Each sample was then reconstituted in 0.1 mL of water and 0.1% formic acid and transferred to an autosampler vial for LC-MS. Each sample was analyzed in triplicate with a 2 μ L injection. Samples were analyzed by the NC State University Molecular Education, Technology and Research Innovation Center

(METRIC) laboratory via LC-MS with a Waters Acquity BEH C18 column according to their established procedures.

Non-Target LC-MS Metabolomics Analysis was performed in addition to the targeted analysis of herbicides. Raw data files were uploaded into Compound Discoverer 3.0 (Thermo Fisher Scientific) and processed using a workflow to find and identify differences between samples. This workflow performed retention time alignment, unknown compound detection and compound grouping across all samples. Elemental compositions were predicted for all compounds and chemical background was hidden using blank samples. Compound annotations were assigned using ddMS2 data in comparison with the mzCloud database (Thermo Fisher Scientific) as well as molecular formula comparisons with eight selected ChemSpider databases (EPA DSSToxCast, EPA ToxCast, MassBank, NIST, Common Pesticide Names, BioCyc, KEGG, HMDB) which were further ranked using the mzLogic algorithm (Thermo Fisher Scientific). A total of 42,762 compounds were identified in the data set. Compounds were filtered in order to exclude background as well as exclude high variance features that are more likely to be attributed to background noise (covariance > 30% in every sample group). Application of these filters resulted in 1,613 compounds, which were utilized for all statistical analysis and data visualization in both Compound Discoverer (CD) and MetaboAnalysyt. Of these 1,613 compounds, 1,080 of were annotated in CD and 106 were considered confident annotations with mzCloud annotation scores higher than 50% (MS/MS fragmentation match to acquired data). An additional 196 of the 1,080 compounds were annotated using mzLogic to simulate fragmentation of ChemSpider IDs and determine the top candidate (MS/MS fragmentation match to theoretical data).

Mass accuracy, retention time, peak shape, and peak area of quality control mix components were monitored throughout the data set to ensure data quality. The percent difference and percent relative standard deviation (%RSD) were calculated from nominal concentrations and laboratory fortified spiked samples. The percent recovery for all fortified spiked samples was above 85%. Eight sample blanks and concentrations of known standards were run in each sample batch. Extraction blanks (provided from EST labs), field blanks, and instrument blanks all contained negligible amounts of atrazine and metolachlor (<0.004 µg/L).

POCIS set 2 was assembled and deployed in collaboration with USGS scientists. The POCIS used in this set were the same as those previously described for sets 1, 3, and 4, except that only one POCIS disk was housed in a canister and deployed at a site rather than three. After the deployment period, the POCIS and field blanks were shipped to the USGS Columbia Environmental Research Center (CERC) in Columbia, MO for extraction. At CERC, each POCIS was individually extracted using 25 mL of methanol (Alvarez, 2010). The sampler extracts from each site and field blank were concentrated using rotary evaporation and nitrogen blowdown, sealed in 1-mL amber glass ampules, and shipped to the USGS National Water Quality Laboratory (NWQL) in Lakewood, CO for analysis. At the NWQL, 90 µL of extract was transferred to an analytical vial and diluted with water (900 µL) and a mixture of internal standards (10 µL) added. Dilution of POCIS extracts was required because most contained co-extracted matrix material that causes suppression of internal standards. Laboratory blank and laboratory-fortified spike samples were prepared using comparable volumes of water and internal standard and processed with the POCIS extracts (Alvarez, 2010).

Water and POCIS extracts were analyzed for 227 pesticide compounds (111 parent compounds and 116 degradates) using direct aqueous injection liquid chromatography tandem

mass spectrometry (DAI LC-MS/MS) (Sandstrom et al., 2016). Samples were analyzed with two injections, one in electrospray ionization (ESI) positive mode and one in ESI negative mode, using two multiple reaction monitoring transitions for each analyte.

Calculation of Time Weighted Average (TWA) Concentration with POCIS. The calculation of a TWA concentration from a POCIS assumes that the sorbent phase is an infinite sink and the accumulation of analytes is linear with time. N_{POCIS} is the mass (μg) of the analyte in the sorbent, t is the exposure time (days) and R_s is the sample rate constant where the concentration in water (C_w) is calculated as:

$$C_w = N_{\text{POCIS}} / R_s \times t$$

All LC-MS data were transformed into ng/POCIS by multiplying ng/mL by the amount of sample injected. R_s values were obtained from peer reviewed publications (Bueno et al., 2009; Dalton et al., 2014; Alvarez et al. 2009; Alvarez 1999).

Statistical Analysis of POCIS. MetaboAnalyst (www.metaboanalyst.ca) 4.0 software (Bellevue, Quebec, Canada) is a comprehensive web-based tool suite utilized to perform metabolic data analysis, visualization and functional interpretation (Chong et al., 2018). Principal component analysis (PCA), partial least squares discriminant analysis and clustering analysis and visualization are all widely used mathematical data interpretation tools within MetaboAnalyst. To better understand herbicide concentration differences among quality control samples, sampling locations and sampling dates PCA was utilized. Using PCA, data was transformed into scores and loadings followed by a singular value decomposition calculation.

RESULTS AND DISCUSSION

TWA of POCIS. POCIS samplers were deployed and retrieved during the active growing season to discern herbicide concentrations in surface water and evaluate their significance relative to restoration efforts of SAV at Lake Mattamuskeet. Atrazine and metolachlor were common herbicide detections among all POCIS sets deployed over the crop production and growing season in waters from the Lake Mattamuskeet watershed (Figure 8). Concentrations of atrazine ranged from 0 – 0.28 $\mu\text{g/L}$ and the greatest concentrations were found between May and July of 2018 (Table 5). Concentrations of metolachlor ranged from 0 – 0.15 $\mu\text{g/L}$ with higher concentrations also found between May and July of 2018 (Table 5). Atrazine was detected at concentrations greater than other sites in the East Canal and West Canal sites. The West Canal sampling location had greater concentrations of metolachlor between the months of April and July. In POCIS set 2 (May - July period), concentrations of 2,4 -D and simazine were measured. Simazine TWA concentrations were relatively low, ranging from 0 – 0.002 $\mu\text{g/L}$ and 2,4-D concentrations ranged from 0 – 0.12 $\mu\text{g/L}$. The highest concentration of 2,4-D was found in the West Canal. PCA verified these differences among sites (Figure 9), as well as differences among quality control field blank samples. West In-Lake samples were also different than the other sites, perhaps due the distance from agricultural inputs and dilution effect of the lake water.

POCIS set 2, which was deployed between May and July of 2018 and analyzed by the USGS detected over 50 compounds with the West Canal site having the greatest number detections (51) and West In-lake having the least number of detections (23) (Figure 10). Similar to the analysis performed by the METRIC laboratory at NC State University for POCIS sets 1, 3, and 4, the detection of compounds in POCIS set 2 was dominated by herbicides, with a smaller number of insecticides quantified. Concentrations of herbicides were also higher compared to

fungicides and insecticides at each site (Figure 11). During this time period, the West Canal site contained the highest concentrations of herbicides followed by the East Canal site leading into Lake Mattamuskeet. The West Canal site also contained the highest concentrations of insecticides (10) and fungicides (9). Herbicide concentrations were subsequently divided among triazines, phenylpyrazoles, chloroacetanilides and others (Figure 12). Triazine herbicides dominated at each site followed by chloroacetanilides. Very low concentrations of phenylpyrazoles were detected at Lake Mattamuskeet ($< 0.001 \mu\text{g/L}$). West In-Lake concentrations were low compared to all other sampling sites.

Berton et al. (2018) studied the quality of surface water in the headwaters of the São Lourenço River in Brazil by utilizing POCIS in 2015 and 2016. Out of the five pesticides studied, atrazine and pyraclostrobin had the greatest concentrations and the greatest detection frequency.. Berton et al. (2018) attributed the moderate soil half-life, medium water solubility, and low adsorption coefficient characteristics that are favorable for leaching, particularly during months with higher precipitation with the results. Maximum concentrations of atrazine reached to $0.94 \mu\text{g/L}$ and were not above Brazilian regulatory standards (Berton et al., 2018). Similar atrazine concentrations were found at Lake Mattamuskeet ($0.28 \mu\text{g/L}$) which is consistent with its environmental profile in respect to soil half-life, water solubility and low adsorption coefficient. On the other hand, PWC predicted environmental concentrations (Chapter 2) differed by more than 200-fold ($63.8 \mu\text{g/L}$).

Poulier et al. (2015) studied the feasibility of POCIS at three sampling sites in an extensive French multi-agricultural watershed in 2012. The authors noted that despite atrazine being banned for use in France since 2003, the Arnac stream contained substantial concentrations of atrazine and its metabolite at the beginning of winter. The authors noted that atrazine

degrades very slowly in water and that its accumulation is plausible in aquifers. Landry et al. (2006) and Morvan et al. (2006) also observed atrazine up to five years after application in an agricultural watershed with concentrations up to 0.43 $\mu\text{g/L}$. Mhadhbi et al. (2019) reported concentrations of atrazine in Tunisia where it is not yet regulatory approved for agricultural use. Similar to our POCIS results, atrazine, simazine and metolachlor were found in the Ruiné river in France utilizing POCIS samplers at concentrations lower than 0.1 $\mu\text{g/L}$ (Pesce et al., 2011).

POCIS samplers seem to serve as the best approach in estimating surface water concentrations in comparison to grab or composite sampling. Grab or composite sampling only offer a brief snapshot of water concentrations. Additional steps are often required to concentrate samples for subsequent chemical analysis (Metcalf et al., 2014). POCIS exhibit negligible loss rates and possess ideal deployment durations to reach equilibrium (Rosen et al., 2018). POCIS also allow small masses of pesticides from episodic release events to be retained in the device by the end of the deployment period (Rosen et al., 2018). The POCIS technique simplifies sampling and sample preparation efforts by eliminating electrical pump requirements, reducing the number of site visits and personnel, and protecting analytes against decomposition during transport and storage (Kot-Wasik et al. 2007). A drawback associated with utilizing POCIS is the necessity of calculating accurate concentrations using the target compound sampling rate (R_s) (L/day) (Dalton et al., 2014; Bueno et al., 2009). For each analyte, the R_s must be determined through laboratory experiments using flow through or static renewal microcosm exposure systems and depend on its inherent properties (Roberts and Bersuder, 2006).

Non-Target LC-MS Metabolomics Analysis. In addition to our targeted analysis of specific herbicides in this study, a non-targeted analysis was performed on the POCIS extracts in an attempt to discover other potentially important compounds that may be influencing SAV

restoration and recovery at Lake Mattamuskeet and to guide future targeted analysis. We found that over one thousand compounds with an identifiable peak area were upload to MetaboAnalyst for statistical analysis. Several additional agricultural and other related chemical profiles were matched including DEET, azoxystrobin, methoxyfenozide, atratone and metolachlor morpholinone.

Azoxystrobin is a commonly used fungicide used to control leaf rust and mildew in wheat. Concentrations of this fungicide were highest at the North Canal and West Canal sites. Van Metre et al. (2017), who deployed one hundred POCIS samplers in small streams across the midwestern US for five weeks during the summer of 2013, found that azoxystrobin was detected in 100% of the POCIS. Another compound, methoxyfenozide, which is an insecticide applied to soybeans and corn was also detected in greater amounts in the West Canal at Lake Mattamuskeet and was also found in approximately 90% of samples in the Midwest (Van Metre et al., 2017).

Interestingly, concentrations of DEET (N,N-diethyl-meta-toluamide) appeared higher in the West-In Lake sample, particularly for the July through September POCIS deployment period. DEET is the active component in insect repellents and was originally developed by the US army in 1946 for personal protection against biting insects and the control of disease transmission (Goa et al., 2020). The detection of DEET is common in water samples collected from urban and agriculturally dominated aquatic environments, sewage treatment plant effluents, groundwater systems, finished drinking water, and even remote areas from potential sources (Costanzo et al., 2007). A potential pathway for DEET to enter aquatic environments is through effluent from handwashing, bathing, washing clothes, or human excretion after application and use. Diffusive sources in agricultural areas such as from septic systems and high recreational areas are also potential pathways, but are not well characterized (Costanzo et al., 2007). DEET

has also presented international challenges while being detected in rivers, lakes and groundwater in Australia, Germany, China, Japan and South Korea (Goa et al., 2020). Recent literature demonstrates that DEET inhibits the growth of algae with an EC_{50} of 4100 $\mu\text{g/L}$ (Goa et al., 2020), and thus may exhibit a similar type of activity on vascular plants like SAV, but additional research is needed on this topic.

In a recent study, duckweed was utilized as a tool for remediating water polluted with triazine herbicides (Panfili et al., 2019). The authors demonstrated that duckweed can be utilized in phytofiltration due to their high proliferation rate, broad adaptability to various conditions and potential resistance (Panfili et al., 2019). These results may indicate that various aquatic plants, if they can survive and grow at Lake Mattamuskeet may offer solutions to SAV restoration and recovery.

In conclusion, results from our in-situ POCIS sampling suggest that 2,4-D, simazine, atrazine and metolachlor are the most commonly measured herbicides in surface waters of the Lake Mattamuskeet watershed. These results seem to reflect the herbicide off-target movement model predictions in Chapter 2 (this document) in that the proper herbicides were selected, but with higher estimated concentrations. Whether these compounds or their measured concentrations would interfere with SAV restoration and recovery at Lake Mattamuskeet is the subject of a larger risk (hazard) analysis for the system (see Chapter 4 in this document). Other chemicals present such as DEET, azoxystrobin, methoxyfenozide, atratone and metolachlor morpholinone could also pose a potential threat to restoration efforts, but additional modeling, monitoring studies, and risk analysis would be needed. Spatial and temporal variations in herbicide detections and concentrations were observed among sampling locations and time periods, indicating a potential concern with localized or pulsed exposures and toxicity to SAV.

Comparisons of TWAC with toxicity estimates of each herbicide is needed to confirm protections for SAV.

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FIGURES

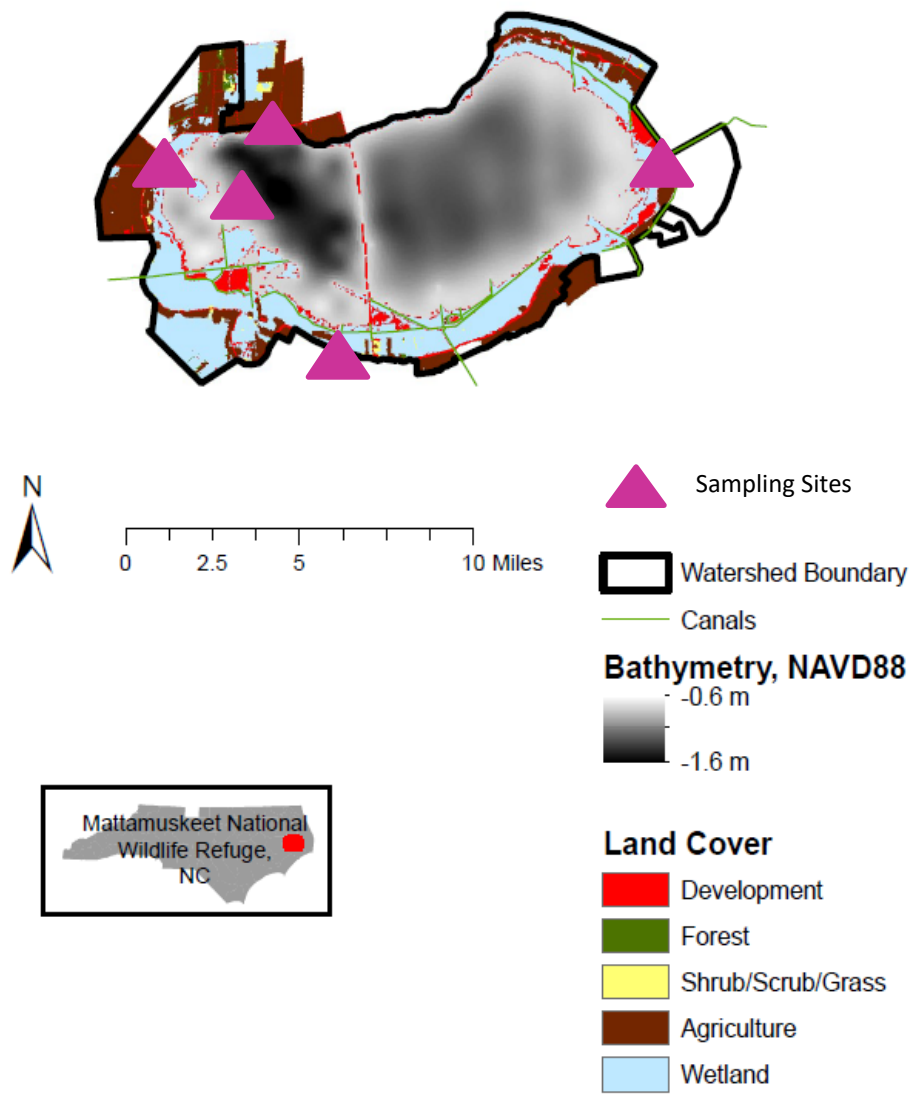
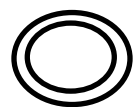
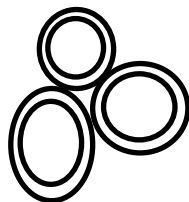


Figure 5. Map of the Lake Mattamuskeet watershed and the five POCIS sampling locations adapted from Moorman et al., 2017.



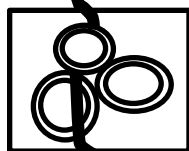
A single POCIS disk



Three POCIS contained with a stainless-steel holder



Floatation device



Three POCIS contained with a stainless-steel holder inside of a paint can with drilled holes



Brick



Figure 6. Diagram (left) and photo (right) demonstrates POCIS configuration for sampling of herbicide concentrations at Lake Mattamuskeet, NC in 2018.

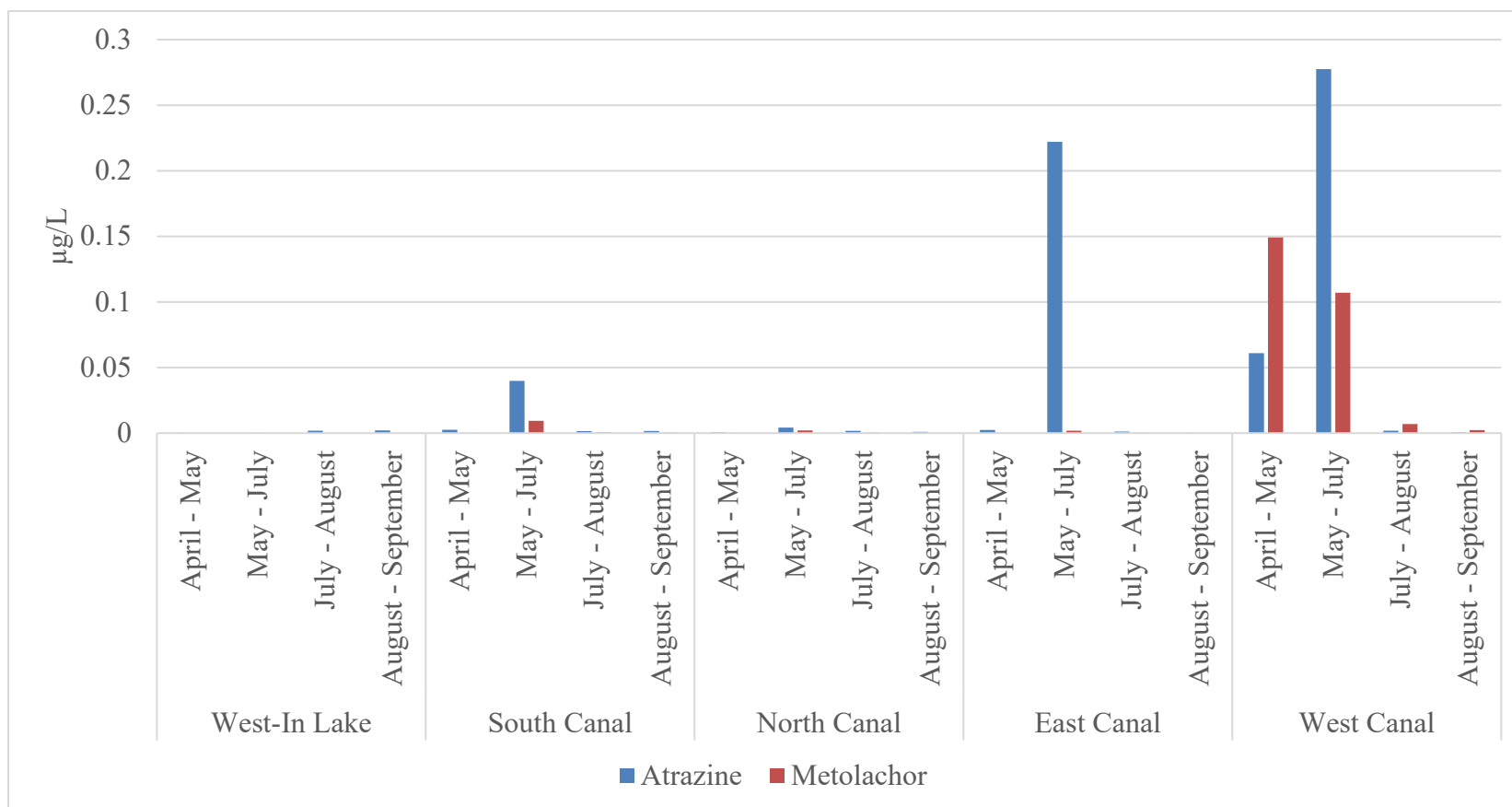


Figure 7. Timed weighted average concentrations ($\mu\text{g/L}$) of atrazine and metolachlor were calculated based on four sequential forty-day POCIS deployment periods at five locations near or in Lake Mattamuskeet, NC in 2018.

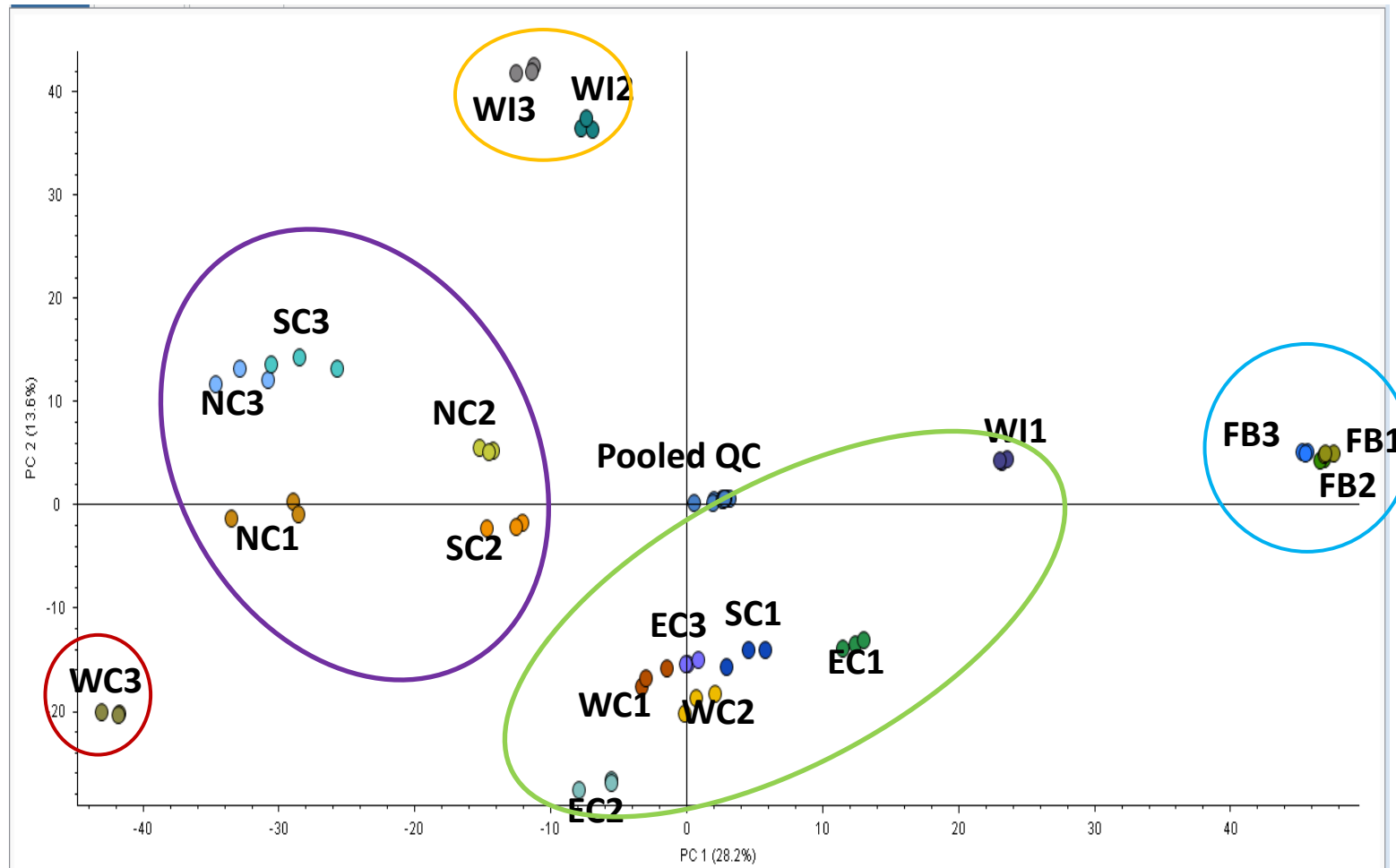


Figure 8. Principal component analysis that demonstrates statistically significant POCIS concentrations among sampling sites. Colored circles indicate site and time grouping similarities. Site abbreviations and POCIS set identifiers for deployments 1, 3, and 4 are defined as follows: WI – West In-Lake, SC – South Canal, NC – North Canal, EC – East Canal, WC – West Canal, FB – Field Blank

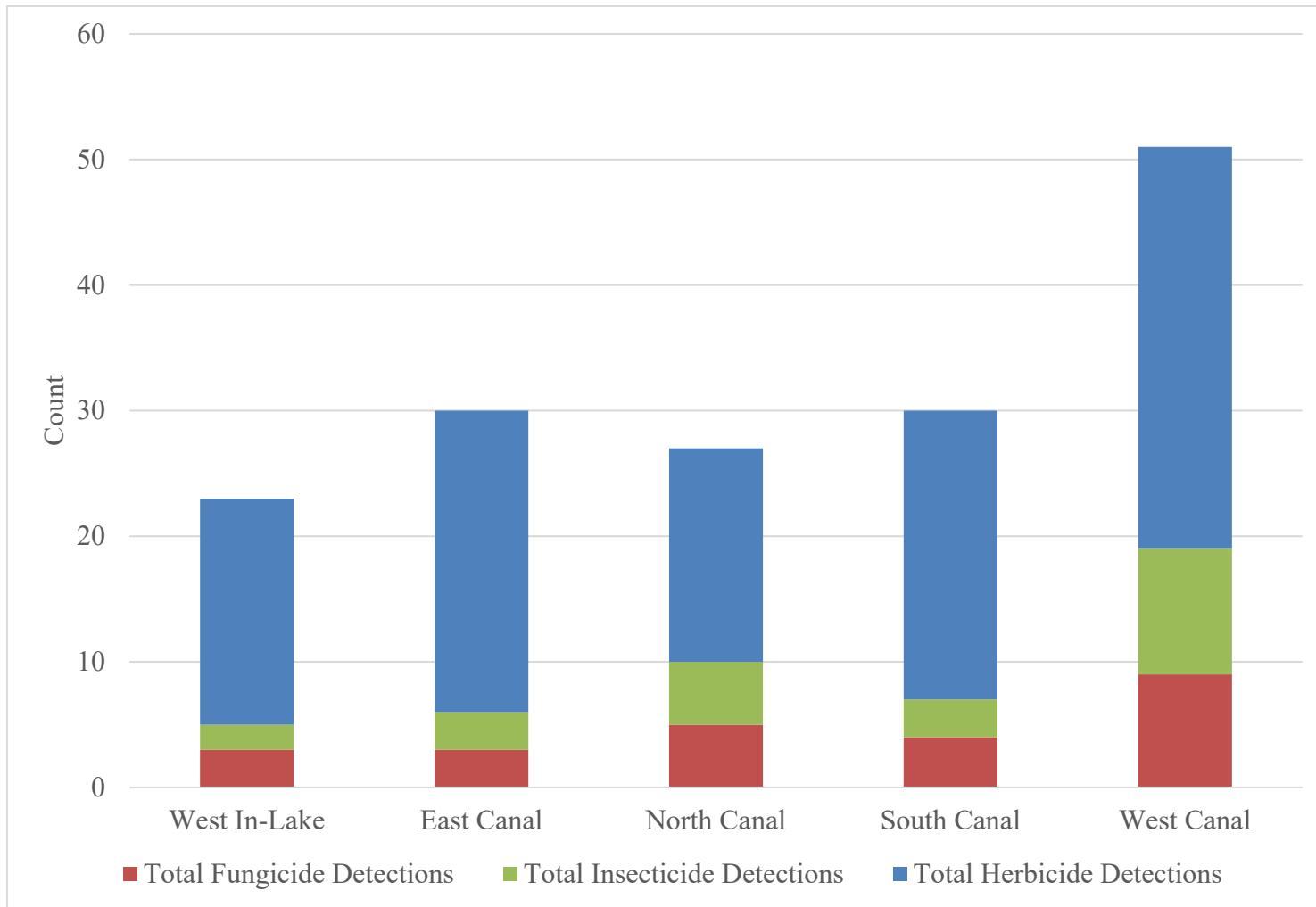


Figure 9. The total number of pesticides (insecticide, fungicide and herbicide) detections quantified by POCIS among sampling sites at Lake Mattamuskeet, NC in 2018.

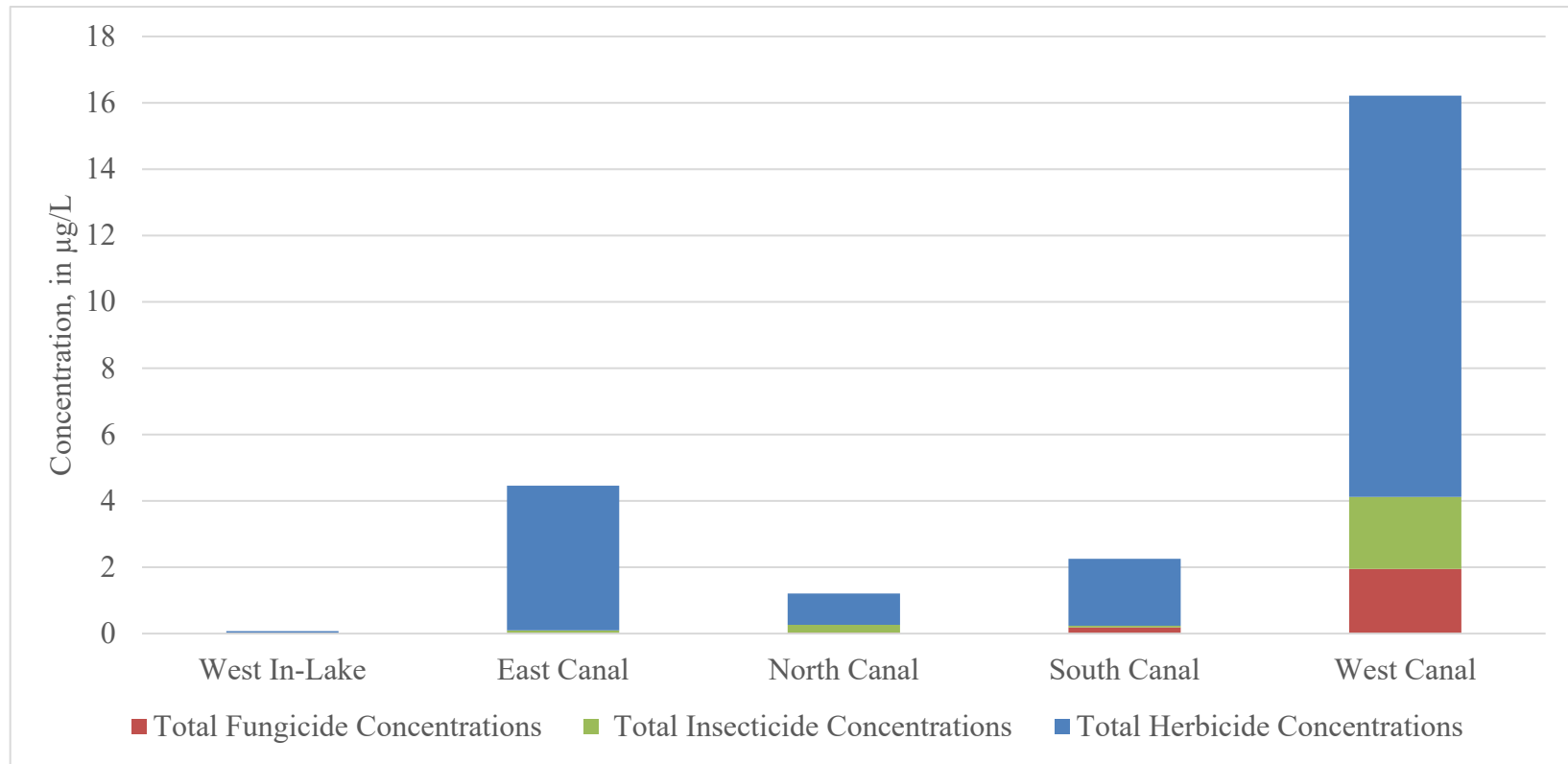


Figure 10. The mathematical summation of pesticide concentrations ($\mu\text{g/L}$) estimated by type for each sampling location at Lake Mattamuskeet, NC in 2018.

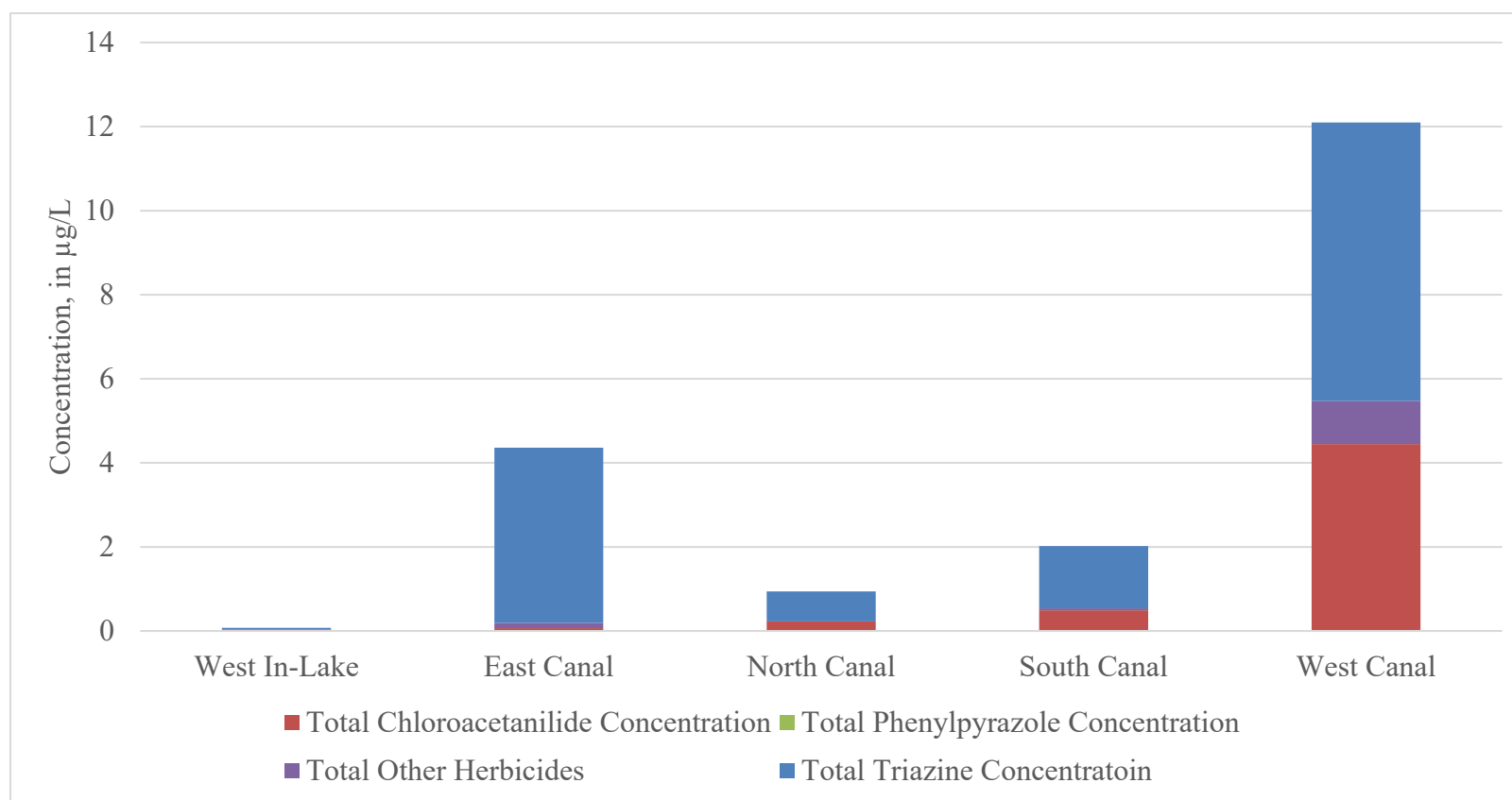


Figure 11. The mathematical summation of herbicide concentrations ($\mu\text{g/L}$) by quantified herbicide class at each site at Lake Mattamuskeet, NC in 2018.

TABLES

Table 3. The sampling locations, coordinates, dates of POCIS deployment and collection, and site identifier used to quantify herbicide transport into Lake Mattamuskeet, NC in 2018.

Site	Site Type	Latitude	Longitude	POCIS Deployment	POCIS Collection
West-In Lake	Lake	35.5058	-76.2539	4/11/2018	5/24/2018
				5/24/2018	7/6/2018 [#]
				7/6/2018	9/7/2018*
				8/16/2018	9/28/2018
South Canal	Canal	35.4483	-76.2279	4/11/2018	5/24/2018
				5/24/2018	7/6/2018
				7/6/2018	8/16/2018
				8/16/2018	9/28/2018
North Canal	Canal	35.537	-76.265	4/11/2018	5/24/2018
				5/24/2018	7/6/2018
				7/6/2018	8/16/2018
				8/16/2018	9/28/2018
West Canal	Canal	35.511	-76.07	4/11/2018	5/24/2018
				5/24/2018	7/6/2018
				7/6/2018	8/16/2018
				8/16/2018	9/28/2018
East Canal	Canal	35.5114	-76.32189	4/11/2018	5/24/2018
				5/24/2018	7/6/2018
				7/6/2018	8/16/2018
				8/16/2018	9/28/2018

* West-In Lake POCIS sampler lost until diver retrieval on 9/7/18.

Observed tractor applying chemical during POCIS collection.

Table 4. A targeted herbicide list for potential transport of herbicides into Lake Mattamuskeet, NC based on their application to the major crops grown in the watershed and predicted environmental concentrations from the Pesticide in Water Calculator (PWC) model (Derived from Chapter 2).

Compound	PWC Estimated 40 Day Pond Concentrations ($\mu\text{g/L}$)			
	corn	cotton	soybean	wheat
2,4-D	30.0	x	x	14.7
Atrazine	63.8	x	x	x
Dicamba	36.6	47.0	32.7	46.1
Flumioxazin	0.1	0.1	0.1	0.1
Fomesafen	x	8.7	5.1	x
Glufosinate	20.9	34.6	26.0	x
Glyphosate	1.4	1.5	0.9	x
Mesosulfuron	x	x	x	3.9
Mesotrione	1.2	x	x	x
Metolachlor	19.9	13.0	9.0	x
Simazine	96.0	x	x	x
Trifloxysulfuron	x	0.5	x	x

<https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/pesticide-water-calculator-version-150-and-152-user>

Table 5. The time weighted average (TWA) concentrations ($\mu\text{g/L}$) of atrazine and metolachlor measured by POCIS at five sampling sites and four-time intervals at Lake Mattamuskeet, NC in 2018.

Location	Date	TWA Atrazine ($\mu\text{g/L}$)	TWA Metolachlor ($\mu\text{g/L}$)
West-In Lake	April - May	0.0	0.0
West-In Lake	May - July	0.0	0.0
West-In Lake	July - August	0.0	0.0
West-In Lake	August - September	0.002	0.0
South Canal	April - May	0.003	0.0
South Canal	May - July	0.040	0.01
South Canal	July - August	0.002	0.0
South Canal	August - September	0.002	0.0
North Canal	April - May	0.0	0.00
North Canal	May - July	0.004	0.002
North Canal	July - August	0.002	0.001
North Canal	August - September	0.01	0.0
East Canal	April - May	0.003	0.0
East Canal	May - July	0.222	0.002
East Canal	July - August	0.001	0.0
East Canal	August - September	0.0	0.0
West Canal	April - May	0.061	0.150
West Canal	May - July	0.278	0.107
West Canal	July - August	0.002	0.007
West Canal	August - September	0.001	0.002

**CHAPTER 4: Comparison of Predicted and Measured Concentrations of Agricultural
Herbicides in an Atlantic Coastal Plain Watershed and their Implications for Restoration
of Submerged Aquatic Vegetation**

ABSTRACT

Submerged aquatic vegetation (SAV) are one of North Carolina's most valuable aquatic resources; a single acre of grass can support as many as 40,000 fish and 50 million small invertebrates. Climatological, biological and environmental events have been identified as leading causes of declines of SAV in temperate and tropical regions of the world. The detection of multiple agrochemicals in the environment has also raised concern that environmental risks from the use of different chemicals within agricultural landscapes may be problematic.

For the risk analysis of agricultural herbicides to SAV performed in this study, we combined maximum modeled predicted environmental concentrations (PECs, from the Pesticide in Water Calculator), maximum measured time weighted average (TWA) concentrations from passive sampling at five sites in the Lake Mattamuskeet, North Carolina watershed, the type of crops grown, and published toxicity data and regulatory standards or benchmarks. When comparing the maximum PECs to regulatory standards, exceedances were observed for 2,4-D, atrazine, metolachlor, and simazine, either through the US EPA Aquatic Life Benchmark for Aquatic Life or through the Canadian Council of Ministers of the Environment CCME Water Quality Guideline for Protection of Aquatic Life. No PECs were exceeded for North Carolina In-Stream Target Values for Surface Waters. Most notably, 2,4-D, atrazine, metolachlor, and simazine all exceeded the CCME Water Quality Guideline for Protection of Aquatic Life. When comparing regulatory guidelines to measured maximum TWA concentrations there were also no exceedances. Similarly, no exceedances were found when comparing the TWA concentrations with the aquatic plant toxicity data. TWA concentrations were between two and six orders of magnitude less than published toxicity values, indicating little to no risk of agricultural herbicide influx to SAV.

INTRODUCTION

Submerged aquatic vegetation (SAV) are one of North Carolina's most valuable aquatic resources where a single acre of grasses can support as many as 40,000 fish and 50 million small invertebrates (Reynolds, 2018). SAV are instrumental in regulating water quality by filtering excess nutrients and sequestering them into their tissues during the active growing season. SAV are also capable of decreasing turbidity by attenuating current and wave energy (Reynolds, 2018). Serving as a shelter for many fish and aquatic invertebrates, SAV are the primary source of food for many species of waterfowl (Reynolds, 2018).

Climatological, biological and environmental events have been identified as leading causes of declines of SAV in temperate and tropical regions of the world (Orth et al., 2006). Threats from global climate change and localized inputs of sediments and nutrients have had demonstrable impacts on the health of seagrasses ecosystems worldwide (Walker et al., 2006). Excess nutrients and sediments are the most significant causes of seagrass declines and result in large losses of acreage each year (Orth et al., 2006). Other noted emerging threats to SAV are the introduction of non-native marine species such as *Halichondria bowerbanki*, *Mya arenaria*, *Alosa sapidissima* and *Zostera japonica* (Carlton 1989) and their acceleration worldwide (Ruiz et al., 2000). At least twenty-eight non-native species have become established in seagrass beds worldwide and sixty-four percent have had negative effects documented (Orth et al., 2006). Lodge et al., (2006) noted that it is impossible to remove a non-native species after its establishment and spread, thus making a non-native permanent species inhabitant. Similar invasive species introductions and adverse ecological consequences have occurred in freshwater ecosystems around the world, including common carp and zebra mussels (Mackie, 1991)

At Lake Mattamuskeet, North Carolina, a large natural lake where SAV has been largely extirpated in recent years (Moorman et al. 2017), efforts are underway to identify, prioritize and conduct monitoring and research to inform management actions that can be implemented to improve water quality and restore SAV (Lake Mattamuskeet Watershed Restoration Plan, 2018). A top research priority of the plan is to understand the potential toxicity of various agricultural chemicals being applied in the watershed to SAV. Continuous water quality monitoring of Lake Mattamuskeet has identified excess nitrogen and phosphorus in the lake that have further contributed to low dissolved oxygen levels, extensive fish kills and nuisance algal blooms (Lake Mattamuskeet Watershed Restoration Plan, 2018). Lake Mattamuskeet was listed on the state 303(d) list of impaired waters due to elevated pH and chlorophyll α (Lake Mattamuskeet Watershed Restoration Plan, 2018; North Carolina Division of Environmental Quality 303(d)). Ongoing studies are being performed that examine local waterfowl impoundments with different water management regimes and the results will help inform the status of current nutrient reduction strategies (Lake Mattamuskeet Watershed Restoration Plan, 2018).

The detection of multiple agrochemicals in the environment has raised concern that environmental risks from the use of different chemicals within agricultural landscapes may be problematic (Kienzler et al., 2016). Crop protection chemicals are highly regulated in most developed economies and undergo standardized environmental risk assessments prior to regulatory approval and subsequent commercial use. Unintentional release of agrochemicals in the aquatic environment can occur through the combination of land use, crop types, agricultural and pest management practices thus resulting in chemical mixtures and rendering single active ingredient risk assessments obsolete (Holmes et al., 2018). Prospective environmental risk assessments calculate the risk of single compounds to aquatic organisms in small edge-of-field

water bodies with limited potential dilution (Holmes et al., 2018). A retrospective assessment of chemicals yields important information that can be used to validate modeling and to determine whether any environmental impairment can be expected from chemicals entering the surrounding landscape (Holmes et al., 2018).

The purpose of this study was to combine modeled predicted environmental concentrations (PECs) of agricultural herbicides with in-situ sampling into a retrospective environmental risk analysis for SAV encompassing the 2018 growing season in the Lake Mattamuskeet watershed of eastern North Carolina. During this retrospective analysis, maximum PECs from the major crop types grown in the watershed (corn, cotton, soybean, winter wheat) were compared to measured time weighted average (TWA) concentrations of herbicides detected during in-situ sampling events with passive sampling technology. Regulatory standards or benchmarks paired with toxicity thresholds from the published scientific literature were also compared to determine whether real-time concentrations of the selected herbicides were conducive to restoring SAV at Lake Mattamuskeet and in the eastern coastal plain.

MATERIALS AND METHODS

Predicted Environmental Concentrations (PECs). The dominant crop types grown in the area surrounding the Lake Mattamuskeet watershed of Hyde County, NC were determined by summarizing annual USDA crop data from 2004 to 2018 (USDA CropScape National Agricultural Statistics Service). Those crops were corn, cotton, soybean and winter wheat. A list of 31 herbicides commonly used on those crops was generated with assistance from agricultural and pesticide experts at NC State University and from Hyde County Cooperative Extension personnel. The PECs were calculated using the Pesticide in Water Calculator (PWC) previously described in Chapter 2. Several parameters were needed to provide estimates for off-

target movement including herbicide physical-chemical data, environmental fate data, application method and timing, crop land, and watershed information. The 2018 North Carolina Agricultural Chemical Manual was utilized for application type and application rates. Product labels were also used to determine targeted crop applications. Meteorological data were downloaded from the US EPA Environmental Modeling Community of Practice for Wilmington, NC due to its proximity to Lake Mattamuskeet. PEC results were reported per crop type for each targeted herbicide (Table 6). From these results, the maximum PEC was selected for each targeted herbicide. This list was then strategically narrowed based on PECs and common detections of herbicides in the scientific literature.

Maximum TWA from Passive Samplers. After deriving the PEC values from PWC, the targeted herbicide list was then further narrowed based on in-situ passive sampling with Polar Organic Chemical Integrative Sampler (POCIS) concentrations of measured herbicides from the 2018 growing season at Lake Mattamuskeet. A TWA concentration was calculated from extracted POCIS and subsequent laboratory analysis (see Chapter 3). For this exercise, TWA values of zero $\mu\text{g/L}$ were removed from consideration and atrazine, metolachlor, simazine and 2,4-D remained.

Toxicity Data from Literature. Aquatic plant toxicity tests using *Selenastrum capricornutum* and *Lemna minor* are commonly conducted in environmental risk assessments to determine the potential impacts of herbicides on primary producers. *Selenastrum capricornutum* and *Lemna minor* are often used as biological monitors in the aquatic environment. Algal tests using the unicellular green algae *Selenastrum capricornutum* were developed in the early 1970s for use in eutrophication assessments and standard operating procedures were subsequently developed for toxicological testing (ASTM 1993a; Fairfield et al., 1997). *Lemna minor* is a floating vascular

plant and is commonly used for testing because of its ease of culture, small size, and rapid reproductive rate (Fairfield et al., 1997; ASTM 1993b). A literature review was performed to collect Median Effective Concentrations at 50% (EC₅₀), No Observed Effect Concentration (NOEC) and the Lowest Observed Effect Concentration (LOEC) for *Selenastrum capricornutum* and *Lemna minor* and results are presented in Table 7.

Regulatory Standard or Benchmark Comparisons. Several aquatic regulatory standards or benchmarks were obtained for comparison to the maximum modeled PECs and maximum measured TWA herbicide concentrations, including the US EPA OPP Aquatic Life Benchmark for Vascular Plants, NC In-Stream Target Values for Surface Waters, the Canadian Council of Ministers of the Environment (CCME) Water Quality Guideline for Protection of Aquatic Life, for Freshwater. The US EPA OPP Aquatic Life Benchmarks are based on toxicity values from EPA reviewed scientific studies and values are used to estimate risk for pesticides and their degradates in ecological risk assessments written in support of pesticide registration (EPA OPP Aquatic Life Benchmarks). For acute vascular plants, the toxicity value is a short-term (less than 10 days) EC₅₀ study with duckweed (EPA OPP Aquatic Life Benchmarks). The North Carolina In-Stream Target Values for Surface Water were designed to protect both fresh and saltwater aquatic organisms to promote biological integrity. The North Carolina In-Stream Target Values are based on a chronic 96-hour LC₅₀ using either the Dungeness crab (*Metacarcinus magister*), opossum shrimp (*Mysis diluviana*) or the northern pink shrimp (*Pandalus borealis*) (NC Division of Water Resources). Values are calculated by multiplying the LC₅₀ from a chronic 96-hour study by a safety factor of 0.01. Similarly, CCME Water Quality guidelines are intended to provide protection of freshwater and marine life from anthropogenic stressors. Guidelines are numerical limits based available scientifically defensible toxicological data and guideline values

meant to protect all forms of aquatic life and all aspects of the aquatic life cycles, including the most sensitive life stage of the most sensitive species over the long term. CCME Water Quality values are also derived from 96-hour chronic studies (CCME Protocol, 2007). Although our PECs encompass a 40-day deployment duration and extend well beyond the 96 hour chronic exposure guidelines, comparisons provide the best estimate of risk to aquatic plants. These results are presented in Table 7.

RESULTS AND DISCUSSION

In this final retrospective risk assessment, maximum PECs, maximum TWAs, regulatory standards and toxicity data from scientific literature were all evaluated and results of this analysis are shown in Table 7. The maximum PECs from the PWC ranged from 19.9 to 96.0 µg/L. When comparing the maximum PECs to regulatory standards, exceedances were observed for 2,4-D, atrazine, metolachlor and simazine either through the US EPA Aquatic Life Benchmark for Aquatic Life or through the CCME Water Quality Guideline for Protection of Aquatic Life. No PECs were exceeded for NC In-Stream Target Values for Surface Waters. Most notably, 2,4-D, atrazine, metolachlor and simazine all exceeded the CCME Water Quality Guideline for Protection of Aquatic Life.

Maximum TWA concentrations from all sampling locations at Lake Mattamuskeet ranged from 0.00015 to 0.28 µg/L, with atrazine having the highest measured TWA at 0.28 µg/L. When comparing regulatory guidelines to measured maximum TWAs, there were no exceedances. Toxicity data were found in the literature for *Selenastrum capricornutum* and *Lemna minor* and no exceedances were observed when comparing the TWAs to these aquatic toxicity data. The TWA concentrations for the herbicides were between two and six orders of magnitude less than toxicity data (Table 7).

Maximum PEC and maximum TWA concentrations differed with predicted concentrations being overestimated by two orders of magnitude. We speculate that our PECs are overestimated concentrations because they are based on a large fixed volume, no flow, pond scenario. POCIS devices placed in low volume, relatively high flowing turbid canals surrounding Lake Mattamuskeet may not best reflect the modeled scenario. In contrast, the PWC also assumes a 10-hectare adjacent agricultural field and Moorman et al., (2017) reported that approximately 4,430 hectares of the 11,890 hectares, approximately 37%, of land draining into the watershed is intensively farmed. In addition, farmlands in low-lying coastal plains are often equipped with pumps that move water rapidly off the landscape directly into the surface waters (Moorman et al., 2017). Given the amount of agricultural land that drains into the watershed (almost 450 times the assumed PWC agricultural field) our TWA concentrations were relatively low at all sampling locations throughout the 2018 agricultural growing season.

Based on this retrospective risk assessment, herbicide off-target movement measured in surface water samples from surrounding agricultural areas seem to pose little to no risk to the SAV restoration efforts at Lake Mattamuskeet given the small sampling window of April – September of 2018. Degradation and loss of vegetation are often symptomology of larger scale concerns (Dalton et al., 2015). Water quality and other anthropogenic inputs further complicate this complex issue and should be addressed to resolve vegetation loss. Dalton et al., (2015) found that nutrient enrichment produced negative effects on riparian and aquatic plant communities in the South Nation River catchment in Canada. Atrazine showed no apparent effect despite its widespread usage (concentrations were $> 0.1 \mu\text{g/L}$ in 14 out of 24 sampling locations). Nitrate was associated with a loss of submerged macrophytes, a shift towards fast-growing emergent and riparian plant species, an increase in non-native species and an overall

reduction in floristic quality (Dalton et al., 2015). Currently, significant management efforts are placed on regulating phosphorus in freshwater systems but the authors of the study in Canada assert that regulating nitrogen is also necessary, if native plant biodiversity conservation is an important goal (Dalton et al., 2015).

In summary, in agreeance with Moorman et al., (2017), our data has demonstrated that targeted herbicide run off poses little to no threat of SAV restoration at Lake Mattamuskeet by combining predicted environmental concentrations with in-situ monitoring during the 2018 growing season in North Carolina. Observations from the Mattamuskeet NWR indicate that SAV declined from the deepest sections of the lake and not at the mouth of the canals. Based on the total planted acres in Hyde County for 2018, applications of herbicides to corn and soybeans presented potential concern according to the PECs from the PWC. In corn, atrazine, and metolachlor had the greatest forty-day pond concentrations whereas soybeans have greater forty-day pond concentrations with pyriithiobac. Among all POCIS deployment periods, atrazine and metolachlor were the most common herbicide detected at Lake Mattamuskeet, with concentrations of atrazine ranging from 0 – 0.28 µg/L. The highest concentrations of atrazine and metolachlor were found between May and July of 2018 when concentrations of metolachlor ranged from 0 – 0.15 µg/L. We also detected concentrations of 2,4 -D and simazine during the May – July POCIS deployment. The simazine time weighted average concentrations were relatively low, ranging from 0 – 0.002 µg/L and 2,4-D concentrations ranged from 0 – 0.12 µg/L. By utilizing the maximum PEC and maximum TWAs, we have presented a conservative worst-case scenario for the targeted herbicides that were modeled and monitored during the 2018 growing season.

This research supports the hypothesis that pesticides are not the driver of SAV declines. By controlling nutrient and sediment influx in the system while removing invasive species like common carp that increases turbidity may lead to a faster holistic recovery (Lake Mattamuskeet Restoration Plan, 2020). Other chemicals such as DEET, azoxystrobin, methoxyfenozide, atratone, and metolachlor morpholinone were detected during a nontargeted analysis and emphasize the findings that other compounds present in surface water could also pose a potential threat to restoration and recovery of SAV at Lake Mattamuskeet. Further research is needed to include a more comprehensive chemical analysis to completely rule out other potential chemical inputs into Lake Mattamuskeet.

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TABLES

Table 6. Estimated forty-day pond concentrations were calculated using 31 herbicides used on dominant crop types grown in the Lake Mattamuskeet watershed of Hyde County, North Carolina utilizing the Pesticide in Water Calculator and environmental fate data obtained from the published literature and herbicide labels. Models were run under assumed 10 hectare adjacent field that drains into a hypothetical 1 ha by 1 m pond. Data derived from Chapter 2.

Compound	US EPA OPP Aquatic Life Benchmark for Vascular Plants ($\mu\text{g/L}$)	Estimated 40 Day Pond Concentrations ($\mu\text{g/L}$)			
		Corn	Cotton	Soybean	Wheat
2,4-D	299.2	30.0	x	x	14.7
Atrazine	4.6	63.8	x	x	x
Dicamba	>3250	36.6	47.0	32.7	46.1
Flumioxazin	0.49	0.1	0.1	0.1	0.1
Fomesafen	210	x	8.7	5.1	x
Glufosinate	1470	20.9	34.6	26.0	x
Glyphosate	11900	1.4	1.5	0.9	x
Mesosulfuron	0.64	x	x	x	0.23
Mesotrione	17.7	1.2	x	x	x
Metolachlor	21	19.9	13.0	9.0	x
Simazine	67	96.0	x	x	x
Trifloxysulfuron	0.24	x	0.50	x	x
Carfentrazone	5.9	0.08	0.12	0.16	0.12
Cloransulam	0.99	x	x	x	0.12
Flumiclorac	>35	x	0.27	x	0.38
Fluometuron	220	x	x	1.17	x
MSMA	104000	x	149	x	x
Pinoxaden	2900	2.3	x	x	x
Pyrithiobac	n/a	x	x	42.4	x
Pyroxsulam	2.57	0.13	0.07	0.14	0.26
Thifensulfuron	1.59	0.35	0.22	x	0.36
Tribenuron	2	0.07	x	x	x
Bentazone	5350	x	46.5	x	159.5
Diclofop	1295	0.08	x	x	x
Paraquat	71	3.7	5.8	6	x
Sethoxydim	210	x	4.6	7.2	6.6
Cyanazine	n/a	x	46.4	x	x
Prometryn	11.9	x	301.5	x	x
Alachlor	2.3	44.3	x	34.8	x
Nicosulfuron	n/a	x	0.4	x	x
Dimethipin	2100	x	x	39.7	x

<https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/pesticide-water-calculator-version-150-and-152-user>; n/a Not available; EPA Environmental Protection Agency

Table 6. (continued)

Compound	CCME Water Quality Guideline for Protection of Aquatic Life, Freshwater (µg/L)	Estimated 40 Day Pond Concentrations (µg/L)			
		Corn	Cotton	Soybean	Wheat
2,4-D	4	30.0	x	x	14.7
Atrazine	1.8	63.8	x	x	x
Dicamba	10	36.6	47.0	32.7	46.1
Flumioxazin	n/a	0.1	0.1	0.1	0.1
Fomesafen	n/a	x	8.7	5.1	x
Glufosinate	n/a	20.9	34.6	26.0	x
Glyphosate	27,000	1.4	1.5	0.9	x
Mesosulfuron	n/a	x	x	x	0.23
Mesotrione	n/a	1.2	x	x	x
Metolachlor	7.8	19.9	13.0	9.0	x
Simazine	10	96.0	x	x	x
Trifloxysulfuron	n/a	x	0.50	x	x
Carfentrazone	n/a	0.08	0.12	0.16	0.12
Cloransulam	n/a	x	x	x	0.12
Flumiclorac	n/a	x	0.27	x	0.38
Fluometuron	n/a	x	x	1.17	x
MSMA	n/a	x	149	x	x
Pinoxaden	n/a	2.3	x	x	x
Pyrithiobac	n/a	x	x	42.4	x
Pyroxsulam	n/a	0.13	0.07	0.14	0.26
Thifensulfuron	n/a	0.35	0.22	x	0.36
Tribenuron	n/a	0.07	x	x	x
Bentazone	n/a	x	46.5	x	159.5
Diclofop	6.1	0.08	x	x	x
Paraquat	n/a	3.7	5.8	6	x
Sethoxydim	n/a	x	4.6	7.2	6.6
Cyanazine	2	x	46.4	x	x
Prometryn	n/a	x	301.5	x	x
Alachlor	n/a	44.3	x	34.8	x
Nicosulfuron	n/a	x	0.4	x	x
Dimethipin	n/a	x	x	39.7	x

<https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/pesticide-water-calculator-version-150-and-152-user>; n/a Not available; CCME Canadian Council of Ministers of the Environment

Table 6. (continued)

Compound	NC In-Stream Target Values for Surface Waters ($\mu\text{g/L}$)	Estimated 40 Day Pond Concentrations ($\mu\text{g/L}$)			
		Corn	Cotton	Soybean	Wheat
2,4-D	60	30.0	x	x	14.7
Atrazine	640	63.8	x	x	x
Dicamba	200	36.6	47.0	32.7	46.1
Flumioxazin	n/a	0.1	0.1	0.1	0.1
Fomesafen	n/a	x	8.7	5.1	x
Glufosinate	n/a	20.9	34.6	26.0	x
Glyphosate	n/a	1.4	1.5	0.9	x
Mesosulfuron	n/a	x	x	x	0.23
Mesotrione	n/a	1.2	x	x	x
Metolachlor	240	19.9	13.0	9.0	x
Simazine	n/a	96.0	x	x	x
Trifloxysulfuron	n/a	x	0.50	x	x
Carfentrazone	n/a	0.08	0.12	0.16	0.12
Cloransulam	n/a	x	x	x	0.12
Flumiclorac	n/a	x	0.27	x	0.38
Fluometuron	n/a	x	x	1.17	x
MSMA	n/a	x	149	x	x
Pinoxaden	n/a	2.3	x	x	x
Pyrithiobac	n/a	x	x	42.4	x
Pyroxsulam	n/a	0.13	0.07	0.14	0.26
Thifensulfuron	n/a	0.35	0.22	x	0.36
Tribenuron	n/a	0.07	x	x	x
Bentazone	n/a	x	46.5	x	159.5
Diclofop	n/a	0.08	x	x	x
Paraquat	n/a	3.7	5.8	6	x
Sethoxydim	n/a	x	4.6	7.2	6.6
Cyanazine	n/a	x	46.4	x	x
Prometryn	n/a	x	301.5	x	x
Alachlor	n/a	44.3	x	34.8	x
Nicosulfuron	n/a	x	0.4	x	x
Dimethipin	n/a	x	x	39.7	x

<https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/pesticide-water-calculator-version-150-and-152-user>; n/a Not available; NC North Carolina

Table 7. A final combination of maximum Predicted Environmental Concentrations (PEC) from the Pesticide in Water Calculator model, maximum Time Weighted Average (TWA) concentrations measured by passive samplers from all five sites at Lake were compiled for Mattamuskeet, NC during 2018 combined with relevant toxicity data for comparison (Median Effective Concentration, EC₅₀; No Observed Effect Concentration, NOEC; Lowest Observed Effect Concentration, LOEC) from the published scientific literature.

Compound	Max PEC (µg/L)	Max TWA (µg/L)	US EPA Aquatic Life Benchmark for Aquatic Plants (µg/L)	CCME Water Quality Guideline for Protection of Aquatic Life, Freshwater (µg/L)	NC In-Stream Target Values for Surface Waters (µg/L)	Toxicity Data from Literature [*]			
						Aquatic Plant	EC ₅₀ (µg/L)	NOEC (µg/L)	LOEC (µg/L)
2,4-D	30.0	0.12	299.2	4	60	<i>Selenastrum capricornutum</i>	41,772	25,000	50,000
						<i>Lemna minor</i>	>100,000	n/a	n/a
Atrazine	63.8	0.28	4.6	1.8	640	<i>Selenastrum capricornutum</i>	235	75	150
						<i>Lemna minor</i>	153	75	150
Metolachlor	19.9	0.15	21	7.8	240	<i>Selenastrum capricornutum</i>	77	38	75
						<i>Lemna minor</i>	343	187	375
Simazine	96.0	0.0015	140	10	n/a	<i>Selenastrum capricornutum</i>	1,240	600	1,200
						<i>Lemna minor</i>	166	75	150

n/a Not available; Data derived from Fairfield et al., 1997; EPA Environmental Protection Agency; CCME Canadian Council of Ministers of the Environment; North Carolina