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Artificial wetlands created in urban areas are ecosystems that provide important services for the management of stormwater, pollutants, and erosion as well as have the potential to support rich biodiversity, unfortunately, they can produce a potent bioaccumulative neurotoxin, methylmercury (MeHg). There exists a need to further elucidate the spatial extent, temporal variation, and biotic export of MeHg from artificial wetlands. In North Carolina, specifically, MeHg concentrations for these systems have yet to be investigated. To address this knowledge gap we compared two artificial wetlands constructed the same year on the campus of the University of North Carolina at Greensboro and conducted a 3-year study measuring MeHg in the surface waters, sediments, and key export organisms for these wetlands. We found that following the installation of the two wetlands MeHg concentrations at these two sites have increased (using campus streams to infer baseline conditions). Mean subsurface sediment samples taken at the wooded wetland site (WS) compared to the open site wetland (OS) were higher in respect to THg concentration and MeHg concentrations, indicating the importance of landscape features in the production and accumulation of MeHg. Mean methylmercury concentrations in pooled Neurocordulia larva samples were observed to be significantly higher at the OS wetland compared to WS . This could be attributed to the presence of organic matter and differences in food chain length that occur between the sites. This information regarding mercury cycling in North Carolina urban areas that can advise future management and construction practices and provide a robust preliminary understanding of how continuing to construct artificial wetlands may enhance MeHg concentrations in urban watersheds.

METHYLMERCURY PRODUCTION IN TWO URBAN ARTIFICAL WETLANDS

by

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DEDICATION

This work is dedicated to my family and friends, who help support me through some of the hardest times while working on this research. Without them none of this work would have been possible. I would also like to thank my advisors and mentors for helping me grow as a scientist, teacher, and mentor over the years and encouraging me throughout the process. I am truly grateful for the team that helped me get to where I am today.

APPROVAL PAGE

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CHAPTER I: INTRODUCTION

1.1 The Wetland Environment

Often referred to as the "kidneys of the Earth", wetlands are unique ecosystems that support a variety of life, provide critical ecosystem services, and serve a vital role in the global landscape (Mitsch & Gosselink, 2015). Yet, they are also now considered the most endangered ecosystem on the planet, mainly due to the extreme loss of continental wetlands (Millennium Ecosystem Assessment (Program), 2005; Verones et al., 2013). It was recently estimated that since the 1700s approximately 21.5% of the world's wetlands have been lost or drained (Fluet-Chouinard et al., 2021; White et al., 2022). Urban development and anthropogenic activities have converted over 3.11 million km^2 of inland wetlands (Ballut-Dajud et al., 2022; Fluet-Chouinard et al., 2021).

1.1.1 Importance and Services

Wetland ecosystems contain approximately a third of the world's threatened and endangered organisms (Murdock, 1994). Wetlands are also some of the most productive ecosystems (by area) in the world, frequently compared to the rainforest and coral reef ecosystems (Mitsch & Gosselink, 2015). In addition to being productive, wetlands provide important ecosystem services. They include being long-term sinks for nutrients, controlling flood water, sequestering carbon, providing ideal habitat for a wide range of organisms, and improving water quality (Smith et al., 2002; Zak et al., 2011). Because of the loss of ecosystem services that has accompanied the loss of wetlands, societal efforts have focused on restoring and creating new wetlands to attempt to mitigate the loss of wetlands and recover the services they provide.

1.1.2 Biogeochemistry and hydrology

Complex biogeochemical processes in wetlands can heavily influence the cycling of potentially toxic trace elements such as mercury (Hg) and its bioavailability within wetland systems. The movement of water can add or remove materials (bringing in nutrients, pollutants, and organic matter), aid in the decomposition of organic matter, and redistribute materials within the wetland and its sediment profile (Roden & Wetzel, 1996). The shallow water table and saturated soils associated with wetlands can provide suitable conditions for the microbial communities that primarily moderates biogeochemical processes (Roden & Wetzel, 1996). The respiratory activity of these microbes influences electron availability and the oxidation and reduction of minerals, in turn, altering the oxidation-reduction potential (ORP) of the wetland sediment (Roden & Wetzel, 2002). Oxygen diffusion in saturated sediments is inefficient and rapidly consumed by microorganisms, providing an anoxic environment which has important implications for microbial production of toxic methylmercury (MeHg) (Ponnamperuma, 1972; Warner et al., 2003). In the oxidized layer of the water column (the uppermost layer), redox values range between +400 to +600 mV and oxygen serves as the dominant electron acceptor. Oxidized forms of ferric iron (FeIII) and sulfate (SO42-) can diffuse downward towards the anoxic sediments and provide substrate for two efficient Hg methylators, sulfate- and iron-reducing bacteria (Eckley et al., 2015; Gilmour et al., 1992). The oxygen-depleted sediments of wetlands are colonized by obligate and facultative anaerobic bacteria (which include all known Hg methylators). At 250 mV, nitrate is reduced to nitrite and can inhibit MeHg production by diverting carbon from sulfate-reducing bacteria to nitrate-reducing bacteria (Shih et al., 2011). At redox potentials +/- 100 mV, the metabolism of iron-reducing bacteria is stimulated as iron is

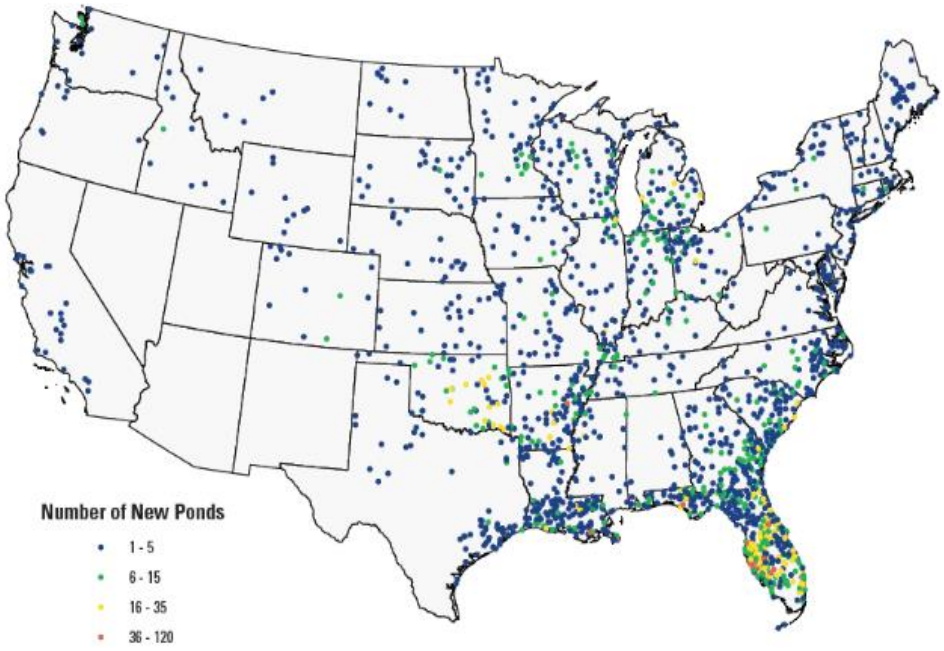
reduced from Fe (III) to Fe(II) and thus the environment becomes more favorable for MeHg production (Mitsch & Gosselink, 2015).

The volume and velocity of water flow is positively associated with the transport of suspended particles and solutes which can supply Hg to the wetland. The longer residence times that characterize wetlands (compared to streams and rivers) create sinks for many of the materials and pollutants that enter their slow waters, such as MeHg (Hall et al., 2008; Windham-Myers et al., 2014); storage of MeHg can then increase exposure to resident fish and aquatic organisms, explaining the role wetlands play as sources of MeHg to the surrounding landscape (Windham-Myers et al., 2014). The movement of subsurface water and the hydraulic connectivity of sediments within wetlands can also influence the movement of water in these systems and thus the movement of particles and solutes.

1.1.3 Wetlands in the urban environment

Across the United States, wetlands have been historically drained, degraded, and destroyed in some manner to allow for population growth, but North Carolina in particular has stood out among the southeastern United States for its net loss of 1.2 million acres of wetlands from 1973 to 1983 (Kramer et al., 1994; Mitsch & Gosselink, 2015). Wetlands have been reported to be present in every county in North Carolina, from the coast to the mountains and thus this state provides an unparalleled opportunity to gain a better understanding of these unique ecosystems (NC State University Cooperative Extension Service, 2010). Recent reports from the U.S. Fish and Wildlife Service show that there continues to be a loss of wetlands nationwide to agricultural land and human expansion each year, with North Carolina losing approximately half a million hectares since 1974 (Dahl, 2011; Hefner, 1994).

Figure 1. New Pond Construction Activity



Note: Map from U.S. Fish and Wildlife Service (Dahl, 2011) shows the number of new freshwater ponds for wetland mitigation and stormwater management constructed from 2004-2009 larger than 0.8ha.

This report also concluded that although considerable wetland loss still occurs, the net outcome was an increase of 207,200 acres due to wetland reestablishment and creation from 2004-2009 in the U.S., mostly occurring in the southeast (Figure 1). While more recent data on the status of wetlands from the U.S. Fish and Wildlife Service has not yet been released, it is still apparent that the effort behind restoration and creation of wetlands has only continued to increase over time. Thus, restored and created wetlands are becoming ubiquitous features of the landscape and the need to gain a deeper understanding of these systems, and if potential drawbacks to their construction exists, has intensified.

1.2 Mercury in the environment

1.2.1 Mercury in the wetland environment

Artificial wetlands are aquatic environments that mimic natural wetland environments. These systems tend to have shallow waters, with frequent drying and rewetting events, high particulate matter inputs via runoff from nearby human-dominated areas, and other key characteristics needed to support the microbial, anaerobic communities that can methylate Hg. Hg methylation processes are predominantly biotic in nature and occur as a metabolic byproduct of sulfate-reducing bacteria (SRB), iron-reducing bacteria (IRB), and methanogens in anoxic environments (Gilmour et al., 2013). In aquatic environments, such as wetlands, most of Hg deposition occurs as wet deposition through precipitation or dry deposition of atmospheric Hg on vegetation that is later introduced into the environment via litterfall during senescence (Duan et al., 2021). Artificial wetlands can also receive Hg inputs from the surrounding urban environment or direct pollutant sources. Once Hg enters a wetland environment it can be methylated in the photic zone of the water column and surface sediment via anaerobic bacteria (Gilmour et al., 2013; Hamelin et al., 2015; Podar et al., 2015). MeHg concentration represents a net concentration that accounts for both production and degradation of MeHg; demethylation can occur simultaneously with methylation activities and both cycles are driven by the wetland's inherent biogeochemistry.

Landscape characteristics can play a role in wetland biogeochemistry and influence Hg cycling and bioavailability (Driscoll et al., 2013; Turnquist et al., 2011). Both constructed and natural wetlands are characterized by the presence of hydrophytic plants, which can influence Hg cycling and storage within the system. High rates of methylation have been found to occur at the root zone of macrophytes, with the highest methylating activity typically occurring at the water-

sediment interface (Goulet et al., 2007; Mauro et al., 2001)). Artificial wetlands share many of the same characteristics that define wetland environments and their biogeochemistry, thus it is reasonable to assume that artificial wetlands are "hotspots" for MeHg productions in the same way that natural wetlands are. Because of the toxicity of MeHg active management of artificial (and natural) wetlands is vital to minimize MeHg bioaccumulation in the food chain (Grigal, 2002; Winder et al., 2020).

1.2.2 Sources, transformations, and fates of mercury in the environment

Recognized as a global pollutant, mercury (Hg) is a naturally occurring, heavy metal. It is emitted by volcanoes, forest fires, crust degassing, and many other environmental phenomena, including human activities; these can contribute to its prevalence since it is mainly produced through combustion events (Ceccatelli et al., 2010). The last several decades have seen dramatic increases in Hg presence which is mainly due to emissions from anthropogenic sources that can include artisanal gold mining, chloroalkali manufacturing (an industrial process used to produce lye), and the combustion of fossil fuels; coal combustion has been recognized as an important source of Hg to the atmosphere (Pirrone et al., 2010). Throughout its cycle, Hg can take on several forms: gaseous elemental Hg(0), oxidized inorganic Hg(II), and organic methylmercury (MeHg).

Hg from the atmosphere is typically in its gaseous form Hg (0) which can travel for long distances for up to a year before depositing in terrestrial and aquatic ecosystems and undergoing further transformation through oxidation-reduction reactions (Fitzgerald et al., 1998; Gregoire & Poulain, 2014). Atmospheric deposition can be either wet deposition in the form of precipitation or dry deposition where Hg(0) or Hg(II) an inorganic form directly enters the landscape (Lamborg et al., 2002). In the environment, Hg can directly enter the terrestrial or aquatic

ecosystems, where it has the potential to be methylated to form MeHg and then bioaccumulate and biomagnify through food webs, prominently affecting organisms at higher trophic levels (Eagles-Smith et al., 2020; Edmonds et al., 2012; Hall et al., 2008).

1.2.3 Mercury bioavailability and bioaccumulation

Environmental factors, such as light availability, water chemistry, and organic matter content, heavily influence Hg biogeochemistry by influencing methylation and demethylation rates and pathways. Bioavailability of Hg depends heavily on its species and the complexes formed with dissolved organic matter (DOM) can have different biogeochemical consequences (Gao, 2022; Miller et al., 2009). Wetlands are typically net MeHg sources, rather than sinks, since they provide suitable habitats for these methylating microbial communities such as methanogens, sulfate-reducing bacteria (SRB), and iron-reducing bacteria (IRB) (Gilmour et al., 1992). These microbial communities thrive in environments high in organic matter and low in oxygen, thus organic matter presence can stimulate microbial activity and increase MeHg presence in wetlands environments.

Once produced, MeHg can bind to organic matter and enter the base of the food web, where it will then biomagnify at each successive trophic level (Morel et al., 1998). Bioaccumulation and biomagnification is thought to be mainly driven by the ambient level of MeHg, which is largely based on the overall methylation potential and the availability of Hg(II) for methylation in a specific ecosystem (Lehnherr et al., 2012). Biodiversity, species richness, and organism life traits (i.e. lifespan) can also influence how much MeHg accumulates in a system, since longer food chains allow more extensive biomagnification of MeHg, leading to the presence of higher MeHg concentrations, such as those observed in top predators (Campbell et al., 2005; Gantner et al., 2009; Winder et al., 2020).

Many of the same microbial communities that can methylate Hg can also demethylate MeHg, such as sulfate-reducing bacteria (SRB), and thus influence mercury bioavailability. Wetland DOC and nutrient content can promote one pathway over the other, such that meso- and eutrophic conditions promote demethylation over methylation and alter Hg bioavailability (Tjerngren et al., 2012; Tsui et al., 2008). Demethylation can be broken into two main pathways: biotic (reductive, oxidative, and methanotrophic) and abiotic (photochemical and dark/chemical) (Barkay & Gu, 2022). The dominant path of demethylation is abiotic photodegradation, in which MeHg can be photolysed into Hg(II), Hg(0), and Hg(S) in aqueous phase (Du, 2019). MeHg photodegradation is mediated by sunlight, specifically UV-A and UV-B wavelengths from 280-400 nm (Du et al., 2019; Hsu-Kim et al., 2018; Li et al., 2010). Historically, there has been more focus on the biogeochemistry of methylation of Mg vs, the demethylation of MeHg. Thus, the understanding of the biogeochemical process governing demethylation or MeHg is still much more obscure than the mercury methylation process.

1.3 Mercury and Methylmercury hazards

Mercury contamination is a significant worldwide concern (Beckers & Rinklebe, 2017). Hg can be toxic in several forms, both inorganic and organic. Inorganic Hg(II) can cause damage to the kidneys and lungs (Clarkson & Magos, 2006). Organic MeHg is typically of greater concern due to its prevalence in the environment, ability to biomagnify in food webs, and high toxicity to humans (O'Connor et al., 2019).

MeHg is known to be a potent neurotoxin that can have critical effects on human health and is regarded as part of the "ten leading chemicals of concern" (Bjørklund et al., 2017; WHO, 2017). MeHg can bioaccumulate and biomagnify in food chains, both terrestrial and aquatic, with organisms at the highest trophic level acquiring the highest concentrations (Fitzgerald et al.,

1998; O'Conner et al., 20019). Aquatic systems are of particular concern because of their potential to be a major source for human MeHg exposure. Long-lived predatory fish can bioaccumulate MeHg to the extent that most of the total mercury concentration is comprised of MeHg posing a significant risk to humans after ingestion (Eisler, 1987). Effects on organisms, mainly birds and mammals, can vary depending on the level of MeHg exposure, ranging from sublethal effects to extensive damage to the nervous, excretory, and reproductive systems and ultimately death (Wiener et al., 2003; Wolfe, 1998). MeHg has also been implicated in reduced fecundity and various central nervous system issues in mammals, including humans that have been exposed (Chan et al., 2003; Karri et al., 2018; Whitney & Cristol, 2018).

The most well-known case of Hg poisoning among humans occurred in Minamata Bay in Japan when an acetaldehyde manufacturing chemical plant discharged high levels of total Hg (THg) and MeHg that contaminated the primary food source for locals, fish, and other seafood products. The results of this event, and the subsequent exposure of humans to MeHg, led to an increase in efforts to understand Minamata disease and how MeHg in the environment affects a wide range of organisms (Harada, 2008). Neurological alterations from the Minamata Bay event were mainly seen among fishermen and families relying on fish that feed in MeHg-contaminated water. Fish, particularly those that live longer and are higher on the food chain, accumulate toxic levels of MeHg, posing a significant risk to humans after ingestion (Farina et al., 2011). Common symptoms reported from the cases of MeHg poisoning include sensory disturbances (ataxia, impairment of speech, deafness, and constriction of visual field), psychiatric symptomatology, tremors, abnormal eye movement, disturbance of taste and smell, and in severe cases, incapacitation or death (Ceccatelli et al., 2010; Kirkpatrick et al., 2015). Significant

adverse effects of MeHg toxicity have also been observed at low doses in some cases (Karagas et al., 2012).

Due to its toxicity, the EPA and other environmental control agencies have set limits to the amount of MeHg that a person should be exposed to daily and what levels are allowed for fish to be safely consumed by humans (National Research Council, 2000). The southeastern US is of particular concern due to the presence of higher Hg levels in fish tissue (many of the areas have fish Hg levels over the advisory limit of 0.3 μg/g wet weight in filet) and it explains why many of these states have issued statewide fish consumption advisories. Yet despite these efforts, individuals living in the United States may be exposed to unsafe levels of MeHg in utero if consumption of seafood increases as it has for many areas around the globe after the pandemic (Liu et al., 2018; Mahaffey et al., 2004; Mandal et al., 2021). Although difficult to quantify, the total burden of MeHg to individuals and society is likely considerable, particularly in regions of the globe where developing nations rely on seafood as their primary source of protein (Mergler et al., 2007). A recent global risk assessment found that climate change will likely create hotspots of "mercury stress" in the central and southern US due to an increase in airborne Hg deposition and methylation under a warming climate (Ferreira-Rodríguez et al., 2021). North Carolina watersheds near the coast (in the coastal plain and sandhills) have significantly higher Hg in fish than those in the piedmont and mountain regions of the state (Sackett et al., 2009); thus, MeHg produced by artificial wetlands can significantly enhance MeHg presence concerning natural (low) background levels and potentially be hazardous for the future of the piedmont landscape, where statewide fish consumption advisories already occur and urban development is rapidly increasing.

1.4 Wetlands in the landscape

1.4.1 Artificial wetlands in the urban landscape

By definition, wetlands are distinguished as transitional areas between terrestrial and aquatic systems; they support life adapted to hydric soils that are covered by water at least partially throughout the year (Cowardin & Service, 1979). This same definition can be applied to the ponds and areas that are constructed to mitigate wetland loss and manage urban stormwater and runoff. Urban ponds have been broadly defined as small (<8 ha or 20 acres) ornamental or recreational freshwater habitats and water bodies that contain shallow waters and retain surface water for most of the year which can occasionally also include residential lakes (Dahl, 2011). Examining the literature reveals a consensus that ponds are considered wetlands (Cowardin & Service, 1979; Dahl, 2011; Oertli, 2009; Strickman & Mitchell, 2018). Artificial wetland is the term applied to wetlands that have been created, either for restoration, recreation, aesthetic, or management purposes. The wetlands include both those ponds used for habitat and for stormwater management, therefore for this study, all the ponds chosen for sampling are considered "artificial" though their purpose may differ slightly; following the example provided by Strickman and Mitchell (2017).

In recent decades, wetland restoration in urban areas has been gaining more attention because of a rising recognition that wetlands can provide a variety of ecosystem services. Such services include, among others, the attenuation of urban heat islands, carbon sequestration, improved water quality, providing habitat for wildlife, and recreation opportunities (Ampatzidis & Kershaw, 2020; Mitsch et al., 2013; Wahlroos et al., 2015; Zhou et al., 2020). The benefits of maintaining wetlands and constructing new wetlands can help mitigate rising challenges to urban environments, such as climate change and water insecurity. At least 55% of the world's

population currently lives in an urban environment and by 2050 it is projected that this number will rise to 68% increasing stress on water quality and the landscape (United Nations, 2019). Another challenge exacerbated in urban areas is climate change, which is more evident by the presence of urban heat islands. The observed cooling effect of wetlands could provide further motivation for creation and construction projects in the years to come to help mitigate the ongoing warming of the planet (Ampatzidis & Kershaw, 2020).

According to the US Fish and Wildlife Service freshwater wetland construction efforts have been focused primarily in the southeastern US (Dahl, 2011). Current records only include wetlands constructed on sites larger than approximately 2 acres. So, the total amount of ponds constructed that could be considered wetlands are likely to exceed projections, particularly in urban areas where most constructed projects are smaller due to space constraints (Dahl, 2011). Restoration efforts seek not only to mitigate the destruction of wetland ecosystems due to urban construction efforts across the globe but also to create new ecosystems to preserve the unique wildlife that occupies these areas and allow for humans and wildlife to share urban landscapes. The ponds constructed, like the natural wetlands, they aim to mimic, serve many purposes from water retention to runoff management (Dahl, 2011).

Once constructed, ponds and artificial wetlands typically receive little management. artificial wetlands present for particular ecological and environmental challenges in urban environments because of their proximity to cities, including altered water regimes, frequent contamination (wastewater, dredging, garbage), and the relatively large populations of exotic species (Alikhani et al., 2021). The management of urban wetlands, both artificial and natural, frequently focuses on these challenges and less on biogeochemical concerns like the production of potentially toxic MeHg.

1.4.2 Artificial wetlands as potential sources of MeHg

Through our evolution, humans have greatly transformed the landscape and wetland construction is just another example of this. Constructing a wetland typically requires the excavation and the alteration of hydrology in the area. The result is a disturbed soil layer, destruction of vegetation, and subsequent sediment within the wetland that is homogenized, compacted, and mineral soil (Bruland & Richardson, 2006; Stolt et al., 2000; Strickman & Mitchell, 2018). The design of most artificial wetlands incorporates features that slow water flow and increase particle retention, whether the main purpose is habitat provision or water management, but ultimately some of the constructed wetlands need to be dredged after approximately 10-15 years post-construction as the sedimentation impedes the functionality of the wetland itself (Kadlec, 2009). These characteristics have various effects on the oxidation-reduction potential, microbial community biodiversity, and activity, as well as the sorption potential between various pollutants and carbon compounds, such as mercury (Stolt et al., 2000; Strickman & Mitchell, 2018).

Wetlands have been implicated as prominent environments for the methylation of Hg to MeHg (Hurley et al., 2002; Strickman & Mitchell, 2018; Tsui et al., 2010) and are often referred to as "hotspots" due to the presence of low dissolved oxygen (DO) and organic matter (OM). The production of MeHg in a wetland is a complex process that can be influenced by several different factors: availability of Hg(II), the amount and quality of organic matter in the sediment (or dissolved organic carbon, DOC, in the water column), the presence of aqueous sulfate and sedimentary sulfide, the balance of mercury methylation and demethylation, the type of microbial community, and physiochemical factors such as temperature that influence the activity

of the microbial community and bioavailability of Hg (Driscoll et al., 2013; Strickman & Mitchell, 2018; Ullrich et al., 2010).

Since artificial wetlands have similar conditions (i.e. anoxic environments) there exists the potential for an increase in the production of MeHg with the creation of new wetlands in urban areas (Chavan et al., 2007; Rumbold & Fink, 2006). As Hg enters the artificial wetland, it has the potential to be methylated and demethylated likely through similar processes present in natural wetlands. This raises a series of questions concerning constructed urban wetlands and how they alter the presence of MeHg in the landscape, and if this increases their potential to put wildlife and humans at greater risk of exposure.

Current studies on mercury cycling in constructed wetlands are limited, but we do know they are capable of supporting environments suitable for the production of MeHg. Strickman and Mitchell (2017) showed novel evidence of in situ production of MeHg from Hg(II) in two types of constructed wetlands in the Greater Toronto area in Ontario, Canada suggesting that artificial wetlands may play an role in MeHg present in the urban landscape. Both wetlands constructed for stormwater management (which tend to lack vegetation) and habitat provision (which are constructed with native vegetation and similar characteristics as natural wetlands) are capable of accumulating and producing MeHg but differ in their observed Hg concentrations and methylation rate (Strickman & Mitchell, 2017). The authors speculate that differences could be due to lower MeHg production potential or higher demethylation, but that further studies are needed to parse out the specific mechanism.

The same group studied found that constructed wetlands at varying stages of maturity differed in their MeHg biogeochemistry. Older wetlands accumulated more OM in their

sediments influencing methylation activity of SRB. This finding is consistent with other findings of soil OM patterns in created and restored wetlands (Bruland and Richardson (2006).

Sinclair et al. (2012) also revealed an interesting pattern in Hg levels of artificial wetlands of different ages. Newly constructed wetlands (≤ 2 years old) had higher MeHg and Total-Hg (THg) concentrations than older artificial wetlands, with the exception of the one wetland in their study that was excavated (all the others were constructed through berming – forming a sediment barrier to retain water). Although these findings contradict findings by Strickman et al (2018b) that mature wetlands had higher MeHg and THg concentrations, it does open the possibility construction practices may play a role in mercury cycling and MeHg production potential.

A study by Fan et al. (2019), aimed at exploring the spatial and temporal distribution in relation to the Hg methylation in various urban constructed artificial wetlands, found that THg concentrations in the four study sites were higher than background levels from lakes and reservoirs (but lower than polluted waters). MeHg was slightly higher than background in comparison, and it was found that MeHg concentration increased with increased water depth of the wetland.

Overall, artificial urban wetlands are understudied and the few published studies have been mainly focused on northern ecosystems in Canada, such as those studied by Strickman et al. (2018b) and Sinclair et al. (2012). There is a need for research in more southern regions (where the growing season and conditions for methylation are present for longer periods of time due to warmer temperatures) and for studies that focus on the mechanisms driving the production and degradation of MeHg in these systems, which remains largely unexplored in order to provide a deeper understanding of the seasonality and transfer of MeHg once produced in these systems.

1.5 Overall Significance and Objectives

In the past several decades, wetland construction in urban areas has increased, both in terms of restoration and stormwater management projects (Dahl, 2011). As urbanization of the landscape continues to meet growing population needs, artificial wetlands will continue to serve as vital tools for flood control, pollutant management, and wildlife habitat provision. As previously mentioned, despite their abundance, these systems are understudied, particularly when it comes to mercury (Hg) cycling and their potential to generate methylmercury (MeHg).

Most research regarding constructed urban wetlands has focused on nutrient cycling or biodiversity, but little is known regarding the potential for these systems to produce MeHg and contribute to landscape-level Hg contamination. The research proposed here aims to further clarify the spatial extent, temporal variation, and mechanism of MeHg production and degradation in urban artificial wetlands of the piedmont of North Carolina, providing novel information regarding mercury cycling in urban areas that can inform future management and construction practices, and provide a robust preliminary understanding of how continuing to construct artificial wetlands may alter MeHg concentrations in urban watersheds.

A review of the literature revealed a significant knowledge gap regarding mercury methylation in artificial wetlands. This lack of knowledge might be problematic given the growing reliance on artificial wetlands in urban and suburban environments for stormwater management and pollution control. The work conducted here was the first to take monthly samples of several artificial wetlands over several years and report concentrations of Hg in these systems in North Carolina. Additionally, this research provided novel information on biogeochemical mechanism driving MeHg concentrations in artificial wetlands. Such knowledge

can be used by land managers and environmental regulators and policy makers to refine current and future construction projects.

The goal of my research was to investigate the role of artificial urban wetlands in the Hg cycle by gaining an understanding of the spatial and temporal variation of Hg and investigating the potential transfer of MeHg from the wetland to the riparian food web (which could lead to implications for terrestrial food webs).

I hypothesize that artificial wetlands in the piedmont of North Carolina will produce elevated levels of MeHg that can then be transferred to aquatic food webs, altering MeHg concentrations in the urban landscape.

I address my hypothesis by attempting to answer three questions:

Question One (Chapter II): How is methylmercury cycling changing through various seasons in artificial wetlands located in urban areas?

Question Two (Chapter II): How does methylmercury cycling change over time as the constructed wetlands mature and become more ecologically stable?

Questions Three (Chapter II): Is there evidence of wetland-derived MeHg export to terrestrial food webs?

CHAPTER II: METHYLMERCURY PRODUCTION IN TWO URBAN ARTIFICIAL WETLANDS

Abstract:

Artificial wetlands created in urban areas are ecosystems that provide important services for the management of stormwater, pollutants, and erosion as well as have the potential to support rich biodiversity, unfortunately, they can produce a potent bioaccumulative neurotoxin, methylmercury (MeHg). There exists a need to further elucidate the spatial extent, temporal variation, and biotic export of MeHg from artificial wetlands. In North Carolina, specifically, MeHg concentrations for these systems have yet to be investigated. To address this knowledge gap we compared two artificial wetlands constructed the same year on the campus of the University of North Carolina at Greensboro and conducted a 3-year study measuring MeHg in the surface waters, sediments, and key export organisms for these wetlands. We found that following the installation of the two wetlands MeHg concentrations at these two sites have increased (using campus streams to infer baseline conditions). Mean subsurface sediment samples taken at the wooded wetland site (WS) compared to the open site wetland (OS) were up to 3x higher concerning THg concentration (118.33 ± 6.0 ng g-1 in WS and 38.63 ± 1.2 ng g-1 in OS). Additionally, sediment MeHg concentrations at WS were 4x higher (0.99 ± 0.10 ng g-1 in WS and 0.24 ± 0.01 ng g-1 in OS), indicating the importance of landscape features in the production and accumulation of MeHg. Mean methylmercury concentrations in pooled Neurocordulia larva samples were observed to be significantly higher at the OS wetland compared to WS (46.91 ± 8.9 ng g-1 and 15.00 ± 3.7 ng g-1 respectively). This is consistent with lower normalized MeHg concentrations, a proxy typically used to predict Hg bioavailability,

observed at WS, and indicative of the influence of organic matter presence and quality. This information regarding mercury cycling in North Carolina urban areas that can advise future management and construction practices and provide a robust preliminary understanding of how continuing to construct artificial wetlands may enhance MeHg concentrations in urban watersheds.

2.1 Introduction

Cities and urban areas around the globe are increasingly relying on artificial wetlands (also known as stormwater management ponds) as convenient solutions to control flooding, pollutants, and erosion while simultaneously providing refuge for wildlife (Knapp et al., 2019; Nivala et al., 2017; Vymazal, 2011; Zhou & Penning-Rowsell, 2021). With increasing urbanization and rising global temperatures, solutions like constructed wetlands become crucial to navigating landscapes with a multitude of sealed surfaces (Knapp et al., 2019; Zhou & Penning-Rowsell, 2021).

Despite their many benefits, artificial wetlands, like other wetlands, produce a bioaccumulative neurotoxin called methylmercury (MeHg) (Sinclair et al., 2012; Strickman & Mitchell, 2017). Little is known regarding the MeHg production capacities of artificial wetlands as they are not often studied, but recent studies have shown that concentrations of MeHg observed are typically lower than those of natural wetlands (Mitchell et al., 2008; Strickman & Mitchell, 2017). Given the highly dynamic nature of wetland biogeochemistry, questions remain regarding MeHg concentrations in southern parts of North America (since most research has focused on northern regions of the continent).

Hg methylation processes are mainly biotic and occur as a metabolic byproduct of sulfate-reducing bacteria (SRB), iron-reducing bacteria (IRB), and methanogens in anoxic

environments (Gilmour et al., 2013). In aquatic environments, such as wetlands, most Hg deposition occurs as wet deposition through precipitation or dry deposition of atmospheric Hg on vegetation that is later introduced into the environment via litterfall during senescence (Duan et al., 2021). Artificial wetlands can also receive Hg inputs from the surrounding urban environment or direct pollutant sources. Once Hg enters a wetland environment it can be methylated in the hypoxic zone of the water column via anaerobic bacteria, along with a variety of other anoxic microhabitats such as aquifers, groundwater, and sediments (Gilmour et al., 2013; Hamelin et al., 2015; Podar et al., 2015). From the environment, MeHg can eventually bioaccumulate and biomagnify through food webs prominently affecting organisms at higher trophic levels (Eagles-Smith et al., 2020; Edmonds et al., 2012; Hall et al., 2008). Organisms emerging out of and moving away from the wetland then have the potential to carry wetland-derived MeHg (within their tissues) to nearby terrestrial landscapes, introducing MeHg to terrestrial species and potentially sensitive organisms such as songbirds (Howie et al., 2018).

Researchers have identified sentinel organisms, various species of Araneae, to gain insight into heavy metal contamination in riparian areas in a manner that offers easy and robust sampling due to the commonality of these organisms (Howie et al., 2018; Larsen et al., 1994; Yang et al., 2016). Riparian spiders have been shown to reflect the transfer of Hg across the aquatic-terrestrial boundary, since they typically feed on emergent insects, such as Odonata larva, that will have bioaccumulated MeHg produced in the aquatic habitats (Cristol et al., 2008; Howie et al., 2018); though it should also be acknowledged that some low levels of MeHg present in spiders will be terrestrially derived through Hg methylation in soils (Tsui et al., 2019). As emerged adults, Odonata larvae can facilitate the transfer of MeHg to spiders, where

terrestrial organisms (such as birds), can then accumulate MeHg (Cristol et al., 2008; Howie et al., 2018; Yang et al., 2016).

The movement of MeHg through biota is heavily influenced by the biogeochemical cycles and soil characteristics of each environment (Mahbub et al., 2017). Artificial wetlands, due to construction practices, have unique biogeochemistry resulting from the disturbed soil layer, destruction of vegetation, and subsequent sedimentation within the wetland that is homogenized, compacted and often comprised of mineral soil (Bruland & Richardson, 2006; Stolt et al., 2000; Strickman & Mitchell, 2018). The design of most artificial wetlands incorporates features that reduce water flow and increase particle retention, whether the main purpose is habitat provision or water management, but ultimately some of the constructed wetlands need to be dredged after approximately 10-15 years post-construction as the sedimentation impedes the functionality of the wetland itself (Kadlec, 2009). These characteristics have various effects on the oxidation-reduction potential, microbial community biodiversity, and activity, as well as the sorption potential for various pollutants, such as mercury (Stolt et al., 2000; Strickman & Mitchell, 2018).

Artificial wetlands also tend to have shallow waters, with frequent drying and rewetting events, high particulate matter inputs via runoff, and other key characteristics needed to support the microbial communities that methylate Hg (Eckley et al., 2015; Feng et al., 2014; Windham-Myers et al., 2014). These systems share many of the characteristics that define wetland environments and their biogeochemistry, thus it is reasonable to assume that like natural wetlands are considered “hotspots” for MeHg productions, created wetlands may be as well and thus management is vital to reduce bioaccumulation in organisms (Grigal, 2002; Winder et al., 2020).

The literature reveals a serious knowledge gap regarding mercury methylation in artificial wetlands, which is concerning given the growing reliance on these systems in urban and suburban environments as stormwater management and pollution control measures. This work will be the first to consistently sample several artificial wetlands over multiple years and report concentrations of Hg in these systems in North Carolina, as well as provide novel information concerning the mechanism driving MeHg concentrations that can be used by land managers and other stakeholders to inform current and future construction projects.

The goal of this research is to elucidate the role of artificial urban wetlands in the Hg cycle by gaining an understanding of the spatial and temporal variation of Hg and investigating the potential transfer of MeHg from the wetland to the riparian food web (which could lead to implications for terrestrial food webs). We will explore this through two main questions: (1) How is methylmercury cycling changing through various seasons and over time in artificial wetlands located in urban areas? And (2) Is there evidence of biotransportation of wetland-derived MeHg to the riparian terrestrial food webs?

For this study, we will use the term "artificial wetland" to describe wetlands that have been created, either for restoration, recreation, aesthetic, or management purposes. The wetlands include both those ponds used for habitat and stormwater management, therefore for this study, all the ponds chosen for sampling are considered "artificial" though their purpose may differ slightly; following the example provided by Strickman and Mitchell (2017).

2.2 Methods

2.2.1 Site Description

In a project led by the Department of Biology at the University of North Carolina at Greensboro (UNCG) campus and Dr. Tom Biebighauser, two sites were chosen on the

university's campus in late 2016. Construction was completed in 2017 with both sites being located within the university's Peabody Park, a landscape comprised of mixed hardwood forests and prairies that are divided by campus roads, paths, and recreational fields. Both sites were also constructed using geotextile pads and a liner (Figure 10).

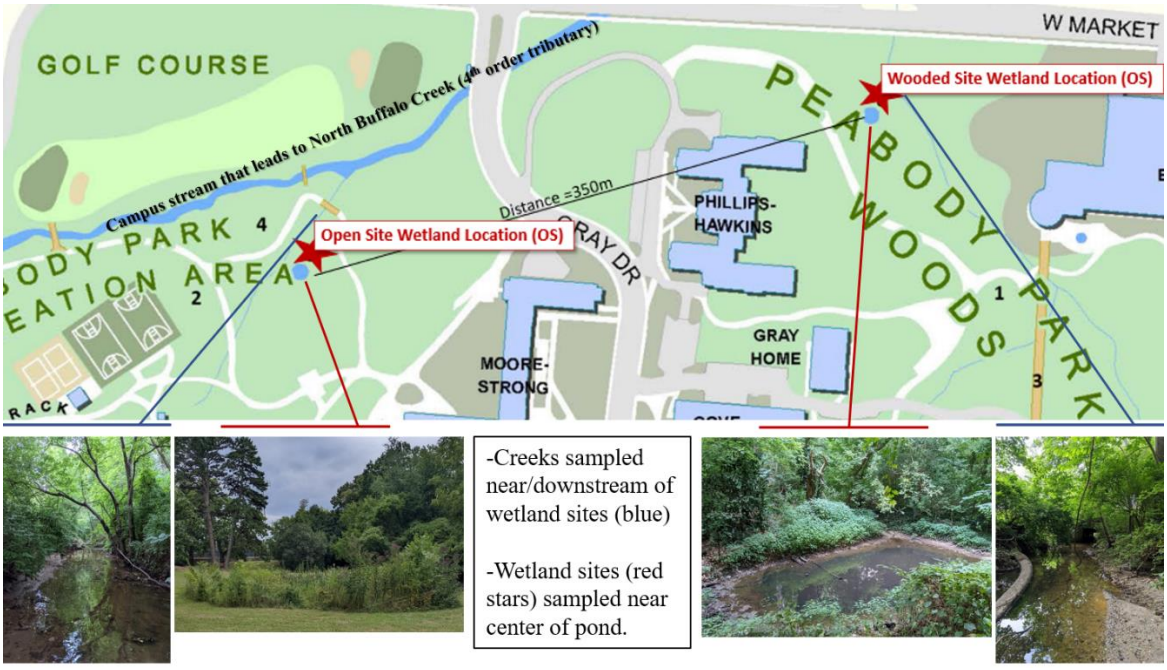
The first wetland will be referred to as the Open Site (or OS) for the remainder of this paper, as it is situated in an open field and resembles a prairie wetland. This wetland is characterized by shallow, permanent water levels, high exposure to UV as it sits in direct sunlight most of the day, and lack of canopy or tree cover. Source water is mainly derived from precipitation as well as some runoff from surrounding lawns that occur at a higher elevation. Approximately two years after construction, this site became colonized by macrophytes, the dominant species being *Typha latiflolia* (Table 2). Other common species found at this site included *Juncus effusus* and *Salix Nigra*. Most of the plants found at OS are the result of strategic plantings that took place shortly after construction (all varieties planted were native to NC piedmont wetlands). Notably, the colonization of emergent macrophytes supports a variety of organisms, particularly many species of amphibians and arachnids at this site. Near the wetland, there is a creek which flows out into a larger stream and eventually connects with North Buffalo Creel (NBC), a 4^{th} order tributary stream in the Reedy Creek Tributary (Figure 2)

The second wetland constructed is located in a forested area of the campus park and has been named the Wooded Site (or WS) and constructed in a manner similar to free water surface (FWS) systems, a common type of constructed wetland for wastewater treatment, in which water flows naturally from an inlet to one or more outlets surrounded by waterproofing to maintain consistent water levels (Gorgoglione & Torretta, 2018). Water primarily enters this system via precipitation and runoff. Runoff has been directed into the wetland via a culvert and during

precipitation events. This wetland can see a considerable increase in water level and movement. The wetland drains into a campus stream that also connects to part of the North Buffalo Creek (NBC) Tributary system (Figure 2); notably, NBC serves as a headwater stream for the Cape Fear River Basin, the state's largest basin (Wilhelm, 2020). Vegetation at this site was primarily composed of mature and immature mixed hardwood species covering a mostly clear understory. Dominant tree and shrub species observed include *Nyssa sylvatica*, *Impatiens capensis*, *Liquidamber styraciflua*, and *Magnolia grandiflora* (Table 2). Likely due to the lack of direct sunlight and deeper water column, this wetland site has not been colonized by any macrophyte species, and many native wetland species that were planted after construction did not thrive.

2.2.2 Sample Collection

Figure 2. Study Site Locations at UNCG



Note: Map of campus wetlands and the nearby streams that were sampled.

All sampling conducted in the field was carried out using trace metal clean methods (clean equipment, gloves, etc.) and samples were immediately taken back to the lab for same-day processing.

Upon sample collection, dissolved oxygen (DO), conductivity, oxidation-reduction potential (ORP), and temperature were recorded in situ using a YSI (Pro2030) and an ExTech ORP meter.

Surface water samples were collected monthly, from October 2017 to October 2020, in two artificial urban wetlands (n=2) at UNCG and in nearby streams (n=2). Water samples were collected into acid-cleaned 500 mL Teflon bottles from selected sites using a 6-foot swing sampler pole to sample the center of the wetland. All samples (n=152) collected were immediately stored on ice, away from sunlight, and brought back to the lab.

Surface sediment samples (1-3 cm in depth) were collected at several locations within the wetland at both OS and WS. Sediment samples were collected several times per year in 2018 with a ramp-up to monthly sampling beginning in early 2019 and continuing till the end of 2020. Initial data showed some seasonal trends in MeHg but could not provide enough information regarding MeHg fluctuations over time, thus a ramp-up in sampling was implemented. Sediment samples were collected (n=20) with a clean spade, placed into new redline zipper bags, and stored on ice and away from sunlight for transport to the lab.

Some dry soil samples were also taken from both wetland sites, about 1.5m from the water's edge, to have a better understanding of Hg levels in the non-flooded parent material.

Along with our main project sites, OS and WS, several other sampling locations were targeted for this project. Two nearby creeks, one that has a direct exchange with WS and one near OS, were chosen in addition to a forest site (with no water body within 150 m).

Invertebrates were sampled along a 100m long gradient, from the nearest wetland to the forested area away from the wetland. Aquatic invertebrate samples were collected approximately every 30-40 m, along the transect. Invertebrates within and around the wetland were targeted, with a focus on riparian spiders and emergent macroinvertebrates (particularly Odonata). Similar species were targeted for each sampling location, with species present along the entire transect taking priority. Terrestrial invertebrates were collected from the wetland and creek banks, with additional sampling occurring at the forested site that was serving as the project's control for MeHg concentration (to gain an understanding of background MeHg levels in a system without a constructed wetland present).

Sampling typically occurred for a period of several hours during the morning. Terrestrial invertebrates were caught using clean powder-free gloved hands and clean forceps or a dip net; some invertebrates were collected via hand-picking. Pitfall traps and a UV light trap were also set the night before sampling to capture a wide variety of invertebrates and gain a better understanding of the biodiversity at each site. Pitfall traps were constructed by burying 16 oz plastic cups so the lip of the cup was flush with the soil surface. The UV trap consisted of a UV light housed in a metal frame over a 5-gallon bucket. The metal funnel under the bulb was directly attached to a clean, large redline zipper bag balanced above dry ice (which was placed directly into the 5-gallon bucket). Aquatic invertebrates were sampled using dip nets and crawfish traps (set the night before sampling occurred).

Dominant Odonata found at both wetland sites was comprised of the genus Neurocordulia (shadow dragons). Dominant Araneae present at the sampling sites differed greatly (likely due to differences in site landscape features) but some recurring genera included: Tigrosa (Wolf Spiders), Dolomedes (Fishing Spiders), and Agelenopsis (Grass Spiders). It

should be noted that finding similar species between sites was particularly challenging and this difference may provide a source of error to the results obtained.

All collected samples were rinsed with DI water to remove the substrate and other debris, before being placed into clean polypropylene centrifuge vials and then into an ice cooler for transport back to the lab. Additionally, on the day of sampling, basic in-situ water chemistry data (via YSI) and water samples were collected using the same methods previously outlined in Chapter II from the creeks and wetland sites. Sediment samples from within the wetland were collected, using the same sampling locations that were used for our previous project (dry soil samples from each site were also taken); sediment was not taken from the creek sites as the creek beds were mostly composed of the rocky substrate. If present, leaf litter samples from the wetland and creek edges and algae were also collected. Leaf litter and algae samples were used to inform baseline trophic levels for $\delta^{15}N$ values.

Previous preliminary biota sampling had occurred briefly in 2018, 2019, and 2020 targeting larval Odonata species so some data was available to compare MeHg concentrations in biota between years.

2.2.3 Sample Processing and Analysis

All sample processing, preparation, and analysis were conducted in an analytical laboratory at UNCG.

Water samples

Following collection, water samples were filtered within 24 hours using pre-baked glassfiber filter paper (Whatman GF/F; nominal pore size of 0.7 µm) and an acid-cleaned all-glass filtration apparatus (cleaned sequentially with dilute HNO_3, dilute BrCl, and rinsed thoroughly). Filtered subsamples for Total-Hg (THg) were transferred to acid-cleaned 125 mL

Teflon bottles and new Hg-free Nalgene PETG (polyethylene terephthalate copolyester, glycol modified) 125 mL bottles for MeHg. A subsample for DOC was also stored in 50 mL polypropylene centrifuge vials to later quantify organic matter content for water samples.

To prepare for THg analysis water samples were digested using an acidic mixture of $KMnO_4$ and $K_2S_2O_8$ in an oven at 60°C overnight following Woerndle et al. (2018) and were then neutralized by an aliquot of NH_2OH. Samples were analyzed by double amalgamation technique and Hg quantification by cold vapor atomic fluorescence spectrometry (CVAFS) via Brooks Rand Model III.

For MeHg analysis, subsamples were preserved with 0.4% trace metal grade (TMG) HCl and kept in the dark, refrigerated, until analysis (Parker & Bloom, 2005). Samples were later distilled to remove matrix interferences by placing weighed aliquot of samples in a Teflon vial in the heat block until approximately all of the samples had been distilled into a cold Teflon receiving vial (for a total of 3-4 hours), which was then ready for analysis. MeHg was quantified using the Brooks Rand Model III CVAFS with an isothermal gas chromatographic separation and pyrolytic unit (Bloom, 1989; Horvat et al., 1993).

Sediment samples

Collected sediment samples were placed into the -20°C freezer the same day and then lyophilized (via VirTis benchtop K) at a later point in time. Before analysis, lyophilized samples were sieved using 1-mm polypropylene mesh fitted into a PVC adapter (both acid-cleaned), and sieved sample was stored in an airtight container away from light until the time of analysis.

To quantify organic matter content, subsamples of sediment were combusted at 400°C in a furnace for 4 hours to obtain loss on ignition (LOI). It was assumed that all of the LOI was attributable to destruction of organic matter. We assumed half of the calculated LOI was organic

C, and then normalized Hg concentrations by dividing by organic C (or 0.5 of OM) since available C can bind to Hg and reduce Hg bioavailability.

For THg analysis, samples were digested via aqua regia as follows (Olund et al., 2004). Approximately 0.2 g of sediment sample was added to an acid-cleaned 40 mL glass vial, followed by 6 mL of TMG HCl and 2 mL of HNO_3. The sample vials were left at room temperature, cold digestion, for a 24 h period. In the following day, 22 mL of 5% BrCl solution was added to the sample vials and the vials were left to digest overnight in a water bath, hot digestion, at 80^{o}C before analysis could begin. For analysis, 0.2 to 2 mL of aliquot of the digested sample were transferred into a glass bubbler containing ~100 mL of nanopure DI water. Then, 200 µL of NH_2OH was added to each bubbler and purging was conducted via Hg-free high-purity N_2 gas for 15 minutes to load the sample Hg onto the gold traps, similar to the method for water samples.

For MeHg analysis, sediment samples were weighed out into 50 mL Teflon distillation vials (~0.1g dw.), to which 30 mL of nanopure water was added, followed by a series of reagents (0.2 mL 20% KCl, 0.4 mL 9 M H_2SO_4, and 0.4 mL 1 M $CuSO_4$) to facilitate the extraction of sedimentary MeHg. Distillation vials containing the sample mixture were then placed in a 140^{o}C aluminum heated block and distilled until only approximately 20% of the sample remained. The distillates (which were transferred to a receiving Teflon vial containing 5 mL of nanopure water) were then ready to be analyzed via the method described earlier (Hammerschmidt et al., 2004).

For both analyses, THg and MeHg, multiple reagent blanks (n=2) were included. We found blanks consistently had undetectable MeHg and very low THg levels in water (<0.04 ng/L and ~0.1 ng/L, respectively). For sediment samples, we also observed consistently undetectable MeHg and low THg concentrations (0.00 ng/L, <0.10 ng/g).

Biota samples

All collected samples were immediately transported to a UNCG analytical lab where they were immediately frozen at -20^{o}C. Prior to invertebrate identification, samples were lyophilized, then detailed information on species and functional feeding groups was recorded (to make it easier to identify potential prey sources). Leaf litter samples were lyophilized and stored in clean ziplock bags, while water and sediment samples were processed and prepared via the methods described above. For all sample analyses, samples were homogenized by finely grinding using a clean agate mortar and then stored in clean 50 mL polypropylene centrifuge tubes. Homogenized subsamples (~0.1 g) were placed into new 15 mL polypropylene centrifuge tubes, to which 6 mL of 4.6 M HNO_3 was subsequently added for MeHg extraction. Samples were then digested for 12 h in a water bath at 60^{o}C and centrifuged at 4,700 rpm for 3 minutes before MeHg analysis (Hammerschmidt & Fitzgerald, 2005). Quantification of MeHg was performed in the same method described earlier (CVAFS).

For stable isotope analyses, subsamples of selected invertebrates, sediment, and litter, were weighed into small tin capsules (approximately mass would be 1 mg, 45 mg, and 2 mg, respectively). Capsules were sealed and placed in a 96-well plate for shipment. Samples were analyzed at the Stable Isotope Facility (SIF) of the University of California Davis campus via an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer (EA-IRMS). Samples were analyzed for both $\delta^{13}C$ and $\delta^{15}N$ in the same sample (along with total C and total N). Specifically, biota samples were analyzed using a PDZ Europa ANCA-GSL elemental analyzer coupled with a PDZ Europa 20-20 IRMS following methods described by SIF (UC Davis Stable Isotope Facility). Sediment samples were analyzed via an Elementar Vario Micro Cube elemental analyzer interfaced to a PDZ Europa 20-20 IRMS, again following methods

described by SIF. Appropriate replicates were used along with 4 laboratory reference materials that had been previously calibrated against international reference materials.

2.2.4 Statistical Analyses

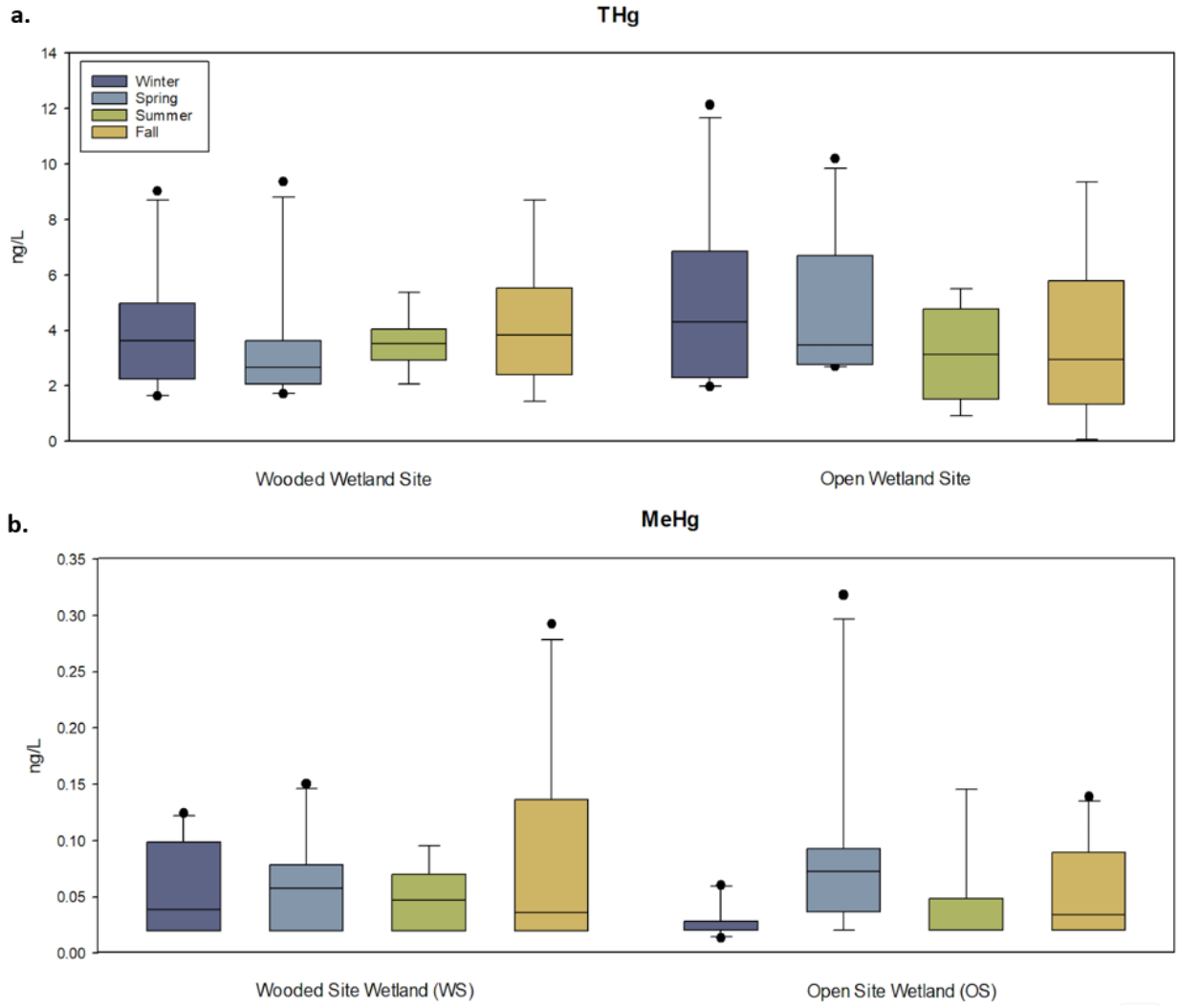
Most statistical analyses and data visualization were conducted in R software version 4.2.1 (R Core Team 2021). I used an alpha level of $P<0.05$ for all analyses. Data for all dependent variables were checked for normality using a Shapiro-Wilk test and log transformations were performed on data that failed. Dependent variables and details for each analysis conducted are presented in Table 4. I used the package lme4 (Bates et al. 2015) to fit Linear Mixed Effect Model (LMEM) with Date as the random term, and site as the fixed term for MeHg concentrations. Results are reported as Mean ± SE. Figures were produced using Sigma Plot 12.5.

2.3 Results and Discussion

2.3.1 Concentrations of Hg observed in water samples

Some seasonal variations of THg and MeHg were observed in filtered surface water samples, with Fall and Winter months having higher concentrations. Water samples did not notably differ in THg concentrations between WS and OS and were highly variable, particularly in OS (4.2 ± 0.44, ng g^{-1} Figure 3a). MeHg concentrations were not statistically significant (not statiscally significant; est = 0.1864, std. error = 0.1750, t-value = 1.065, Table 4) and were also similar between sites (0.06 ± 0.01 ng L^{-1} and 0.05 ± 0.01 ng L^{-1} respectively, Figure 3b); although they were consistently somewhat higher in WS. Water samples were likely influenced highly by precipitation events, making it more difficult to observe any specific trend (Wang et al., 2012).

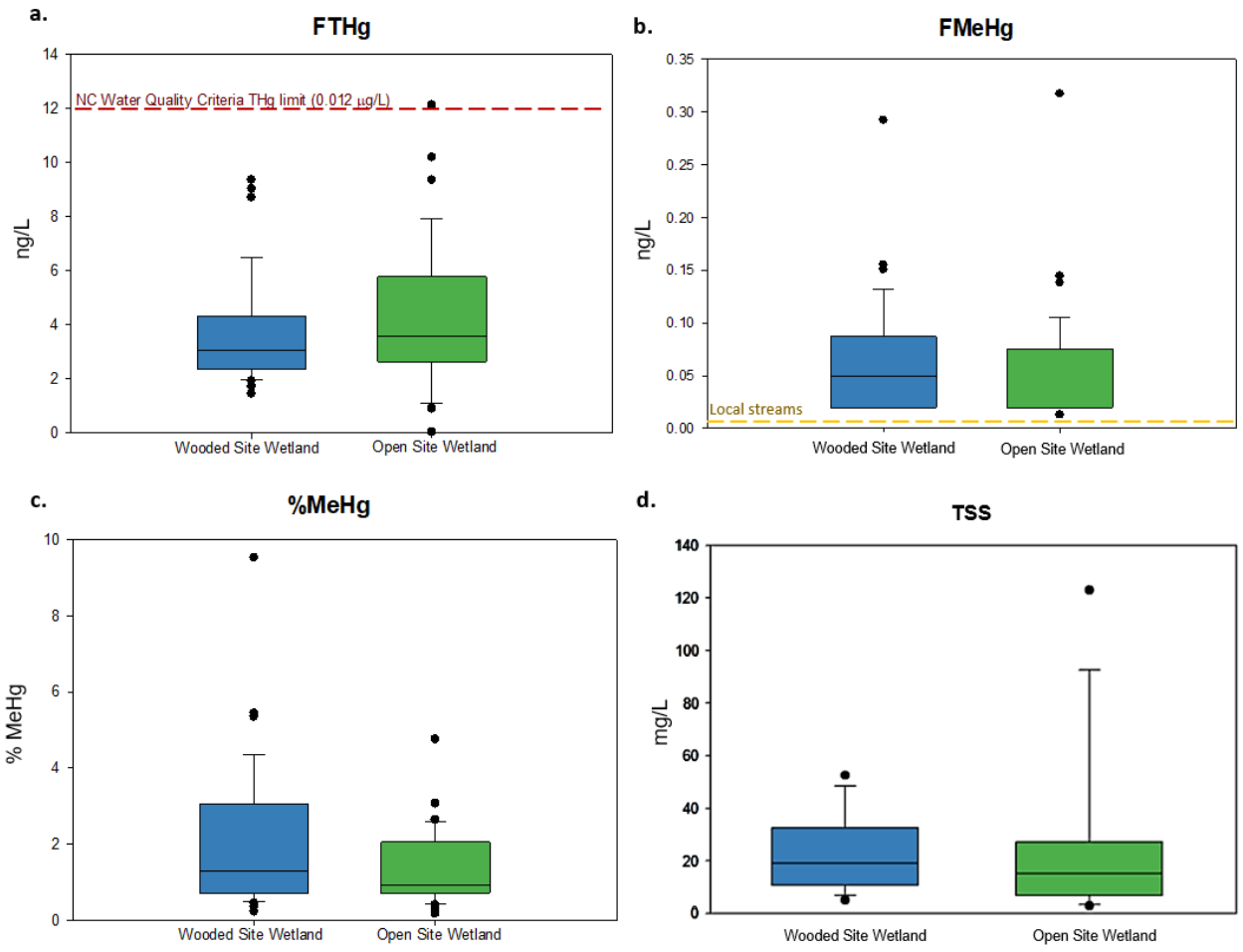
Figure 3. Seasonal Water Hg Concentrations



Note: Comparison between two wetland sites (WS and OS) of a) total-mercury (THg) and b) methylmercury (MeHg) concentrations in surface water across various seasons. All data are presented as median (central line) with outliers plotted as points that lie outside of the 10th and 90th percentiles. Seasons were divided into Winter (Dec, Jan, Feb), Spring (Mar, Apr, May), Summer (Jun, Jul, Aug), and Fall (Sept, Oct, Nov); data from all years was aggregated into the specific seasons defined.

Overall, mean surface water samples (filtered) varied greatly throughout the study period, likely due to the influence of precipitation and light availability (Wang et al., 2020). MeHg and THg concentrations were similar (not statiscally significant; est = 0.1961, std. error = 0.1433, t-value = 1.369, Table 4) between the wooded wetland site (WS) and the open wetland site (OS) (Figure 4a-b), which is likely due to the proximity of these sites and similarity in size (Strickman & Mitchell, 2017; Wang et al., 2020). Streams sampled on campus (near each wetland site) had MeHg concentrations typically below the detection limit (0.04 ng/L) and lower than those observed within the study sites (Figure 4a). This data is consistent with other streams sampled in the area in 2015 summer (Tsui et al., unpublished) and provides adequate background information for Hg levels on campus (before wetland installation). Both wetland sites have concentrations below the North Carolina Water Quality Criteria THg limit of 12 ng/L(NCDWQ, 2012), unlike natural wetlands within the state which have reported concentrations of 8.07 ng L-1 (Figure 4b, Table 1). %MeHg (ratio of Hg present as MeHg) and TSS (total suspended solids) did not differ significantly between sites, though both variables were slightly higher at WS (Figure 4 c-d) where organic matter content and leaf litter decomposition activities are more prevalent.

Figure 4. Filtered Water Sample THg, MeHg, %MeHg, and TSS

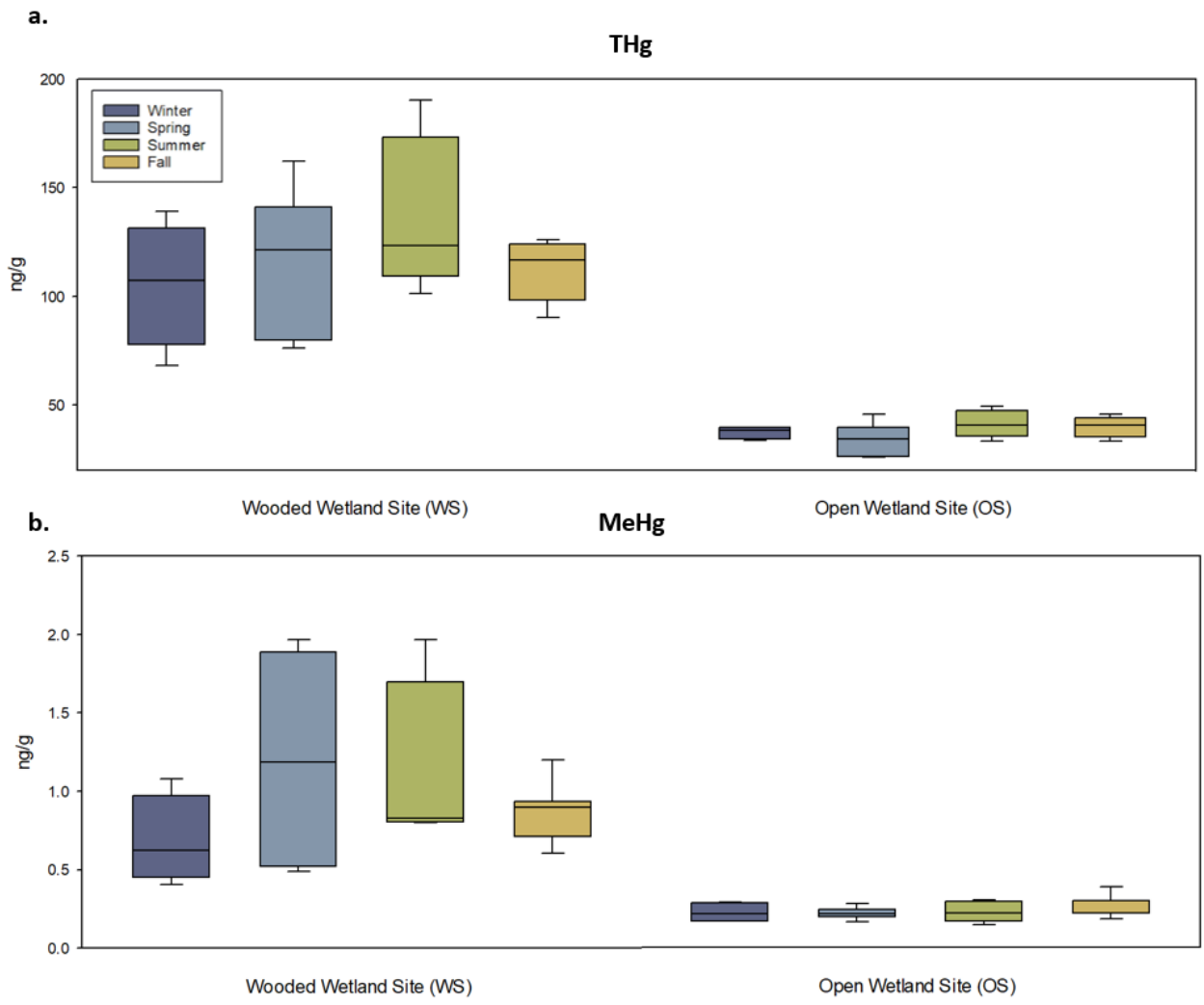


Note: Filtered surface water samples compared between wetland sites for a) total-mercury (THg) concentration, b) methylmercury (MeHg) concentration, c) the percentage of mercury present as methylmercury for the study period, and d) total suspended solids (TSS). The horizontal lines indicate the concentration of THg for local streams (yellow) and the NC water quality criteria limit set by the NCDEQ (red), respectively. All data are presented as median (central line) with outliers plotted as points that lie outside of the 10th and 90th percentiles; data from every year collected was aggregated.

2.3.2 Concentrations of Hg observed in sediment samples

Seasonal variations of THg and MeHg were also observed in sediment samples, with concentrations of MeHg peaking during the warmer months (spring and summer) and falling during the cooler months (not statiscally significant; est = 1.34841, std. error = 0.11072, t-value = 12.18, Table 4). Total-mercury (THg) concentrations show distinct differences between sediment samples from the WS and OS sites across all seasons (118.33 ± 6.0 ng g-1 and 38.63 ± 1.2 ng g^{-1} respectively, Figure 5a). Similarly, methylmercury (MeHg) data from WS sediments were much more elevated (0.99 ± 0.10 ng g^{-1} dry wt., Figure 5b) compared to OS (0.24 ± 0.02 ng g^{-1} dry wt., Figure 5b).

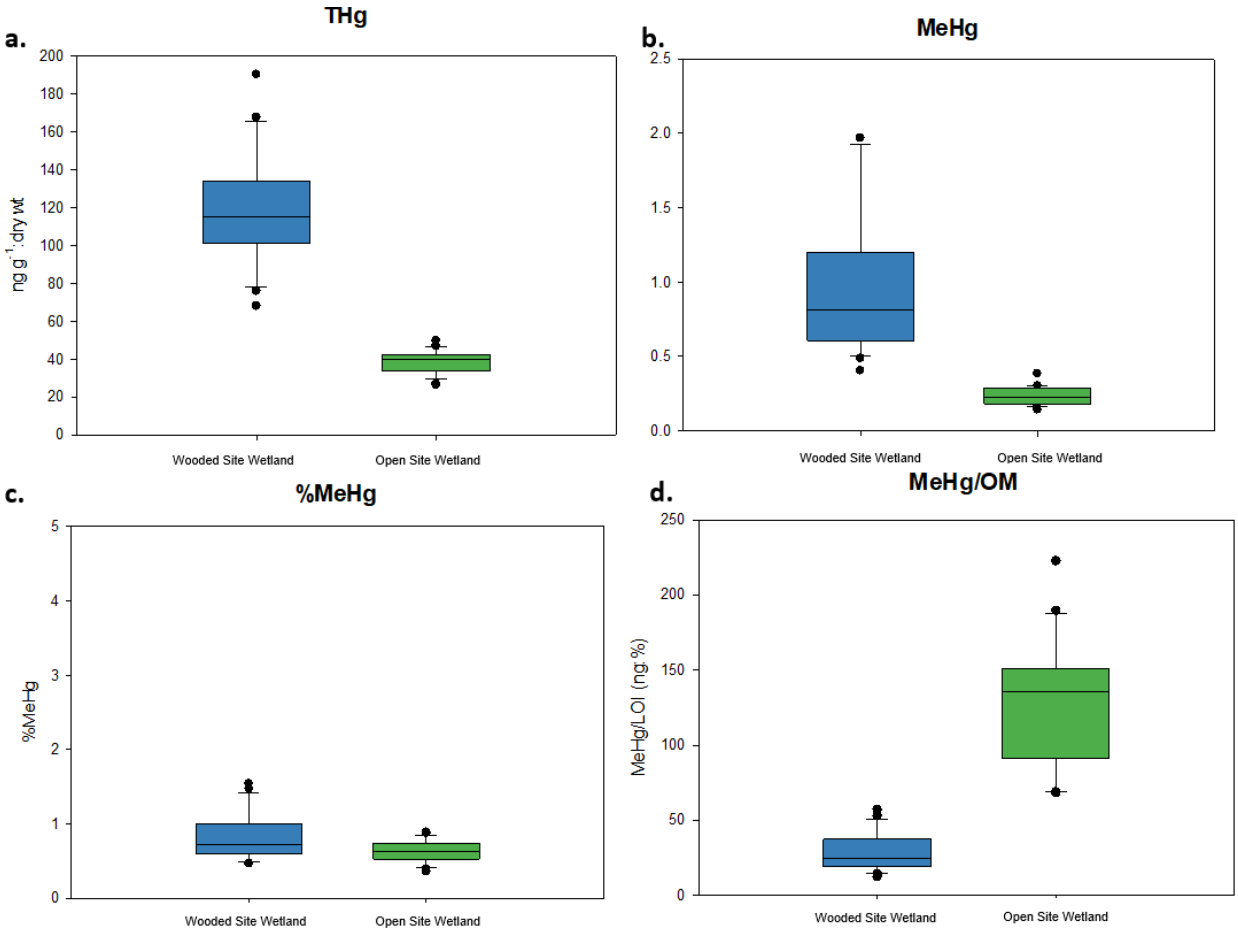
Figure 5. Seasonal Sediment Hg Concentrations



Note: Comparison between two wetland sites (WS and OS) of a) total-mercury (THg) and b) methylmercury (MeHg) concentrations in sediment samples across various seasons. All data are presented as median (central line) with outliers plotted as points that lie outside of the 10th and 90th percentiles. Seasons were divided into Winter (Dec, Jan, Feb), Spring (Mar, Apr, May), Summer (Jun, Jul, Aug), and Fall (Sept, Oct, Nov); data from all years was aggregated into the specific seasons defined.

Sediment samples (taken at 1-3 cm depth) were up to 3x higher with respect to THg concentration (118.33 ± 6.0 ng g^{-1} in WS and 38.63 ± 1.2 ng g^{-1} in OS) and 4x regarding MeHg concentrations (0.99 ± 0.10 ng g-1 in WS and 0.24 ± 0.01 ng g-1 in OS) over the 3-year study period (Figure 6). Concentrations of THg and MeHg were higher in the wooded wetland site (WS) than in the open site wetland (OS) (not statiscally significant; not statiscally significant; est = 1.34839, std. error = 0.10455, t-value = 12.90, Table 4), a pattern that was observed over time as well (Figure 5). The mean percentage of THg present as MeHg (%MeHg) can elucidate the efficiency of the conversion of inorganic Hg to MeHg and also considers the long-term MeHg accumulation in sediment samples (Wang et al., 2020) and did not vary greatly between WS and OS (0.82 ± 0.06% and 0.63 ± 0.03%, Figure 6c). Methylmercury concentrations normalized to organic matter content, a proxy typically used to predict Hg bioavailability (Bryan & Langston, 1992) show lower bioavailability for WS (Figure 6d), which is consistent with the trends observed in organic matter content for the same study site (Figure 11) emphasizing the importance of organic material controlling MeHg availability.

Figure 6. Sediment Sample THg, MeHg, %MeHg, and TSS



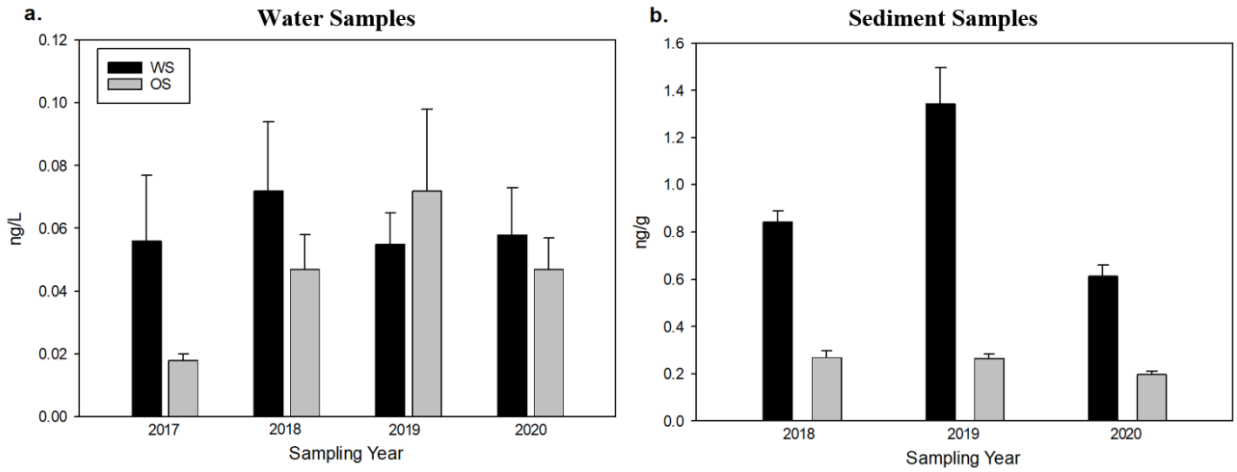
Note: Sediment samples compared between wetland sites for a) total-mercury (THg) concentration, b) methylmercury (MeHg) concentration, c) the percentage of mercury present as methylmercury for the study period, and d) MeHg normalized to organic matter (MeHg/OM). All data are presented as median (central line) with outliers plotted as points that lie outside of the 10th and 90th percentiles; data from every year collected was aggregated.

2.3.3 Concentrations of Hg over time

OS water samples did show a slight increase from 2017 to 2019, but this pattern did not continue into 2020 (Figure 7a) and overall the data was not significantly different between years (est = 0.1961, std. error = 0.1827 , t-value = 1.074 , Table 4). This trend could potentially be

explained by an increase in macrophyte colonization, particularly *Typha latiflolia*, which has been found to increase Hg accumulation and methylation (in plant roots and periphyton) by stimulating microbial biodiversity, such as SRB (Gentès et al., 2013). In general, 2020 MeHg concentrations for both sediment and water samples were slightly lower than samples collected in earlier years at both wetland sites. Yearly mean sediment MeHg concentration did not show any specific trends (increase or decrease) over time (Figure 7b; not statiscally significant, est = 1.34834, std. error = 0.08591, t-value = 15.694, Table 4).

Figure 7. Water and Sediment MeHg Concentrations over Time



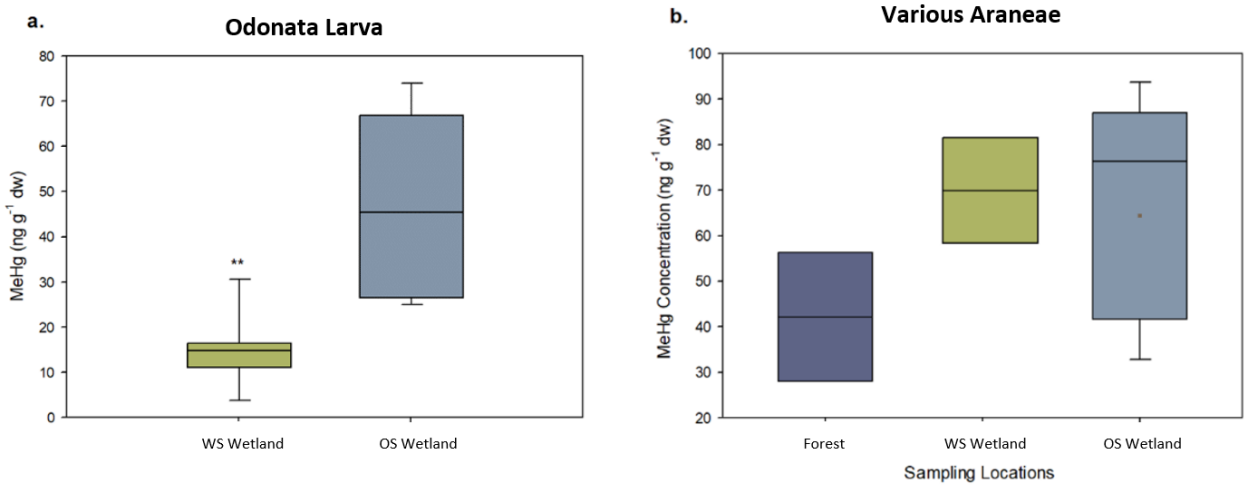
Note: Mean MeHg concentrations plotted over time (sampling year) for a) water samples and b) sediment samples collected from both wetland sites. No significant trends were observed over time (P-value>0.05). OS aqueous MeHg concentrations slightly increased from 2017-2019. All data are presented as means with mean standard error displayed as error bars.

2.3.4 Concentrations of Hg observed in biota samples

Mean MeHg concentrations in pooled dragonfly or Odonata (genus *Neurocordulia*) larva samples were observed to be significantly higher at the OS wetland compared to WS (46.91 ± 8.9 ng g^{-1} and 15.00 ± 3.7 ng g^{-1} respectively; F-value= 15.07, P-value= 0.00255, Table 4; Figure

8a). Higher concentrations of MeHg in OS biota support earlier data regarding MeHg bioavailability at this site (Figure 6d) and are likely influenced by the quantity and quality of organic matter in the sediment at WS (Hill et al., 2009). Araneae samples (consisting of *Tigrosa*, *Dolomedes*, and *Agelenopsis*) collected from around the two wetland sites and a nearby forested site showed variable mean MeHg concentrations that were not significantly different between sites (Forest: 42.18 ± 10.7 ng g^{-1}, OS: 67.79 ± 10.9 ng g^{-1}, WS: 69.96 ± 11.5ng g^{-1}; F-value= 0.7088, P-value= 0.5811, Table 4; Figure 8b). Concentrations observed at the open site wetland (OS) were slightly higher than at the other sampling locations (but contained significant variation compared to the other sites), showing a similar pattern to the Odonata (Dragonfly) larva (Figure 8b). Variation in MeHg concentration at OS is likely due to several factors, including the size, age, and feeding ecology of the sampled organisms (Clayden et al., 2014).

Figure 8. Dragonfly Nymph and Spider MeHg Concentrations

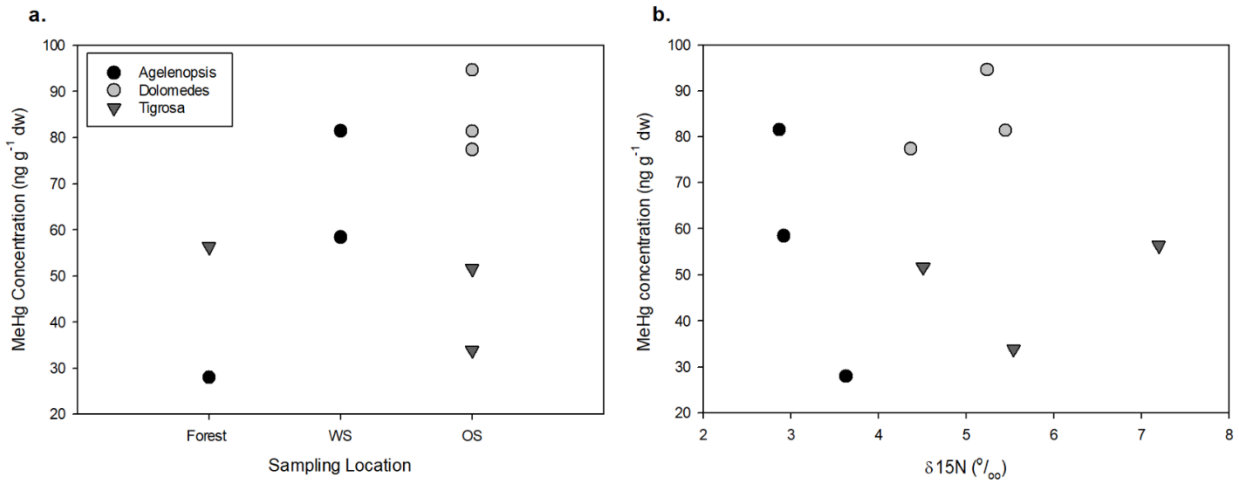


Note: Pooled methylmercury concentrations observed in a) Odonata (*Neurocordulia)* nymphs collected from both wetland sites and b) various riparian Aranea specimens over three consecutive summers. Concentrations in Odonata larva collected from the open site wetland (OS) were significantly higher ($P<0.01$) than those collected from the wooded site wetland (WS).

Araneae specimens in the OS site were slightly higher than the WS wetland and Forest site. All data are presented as median (central line) with outliers plotted as points that lie outside of the 10th and 90th percentiles.

Mean MeHg concentrations between the three sampling locations (Forest, OS, and WS) show slightly higher concentrations at both wetland sites (Figure 9a), following patterns frequently seen in the literature where biota near wetland sites have elevated MeHg (Chasar et al., 2009; Hurley et al., 2002; St. Louis et al., 1994). $\delta^{15}N$ measured for specimens collected show that samples collected at the WS site, mainly *Agelenopsis*, had lower $\delta^{15}N$ isotopic concentrations, while *Tigrosa* (dominant genera found at the Forest and OS site) had had higher $\delta^{15}N$ isotopic concentrations (Figure 9b). Both Agelenopsis (grass spiders) and Tigrosa (wolf spiders) are common spiders and frequently used as sentinel organisms for metal pollution, although similar in size they differ slightly in hunting strategy which could explain the differences seen in Figure 9b (Yang et al., 2016). *Dolomedes* specimens were only found at the OS site but have a similar hunting strategy to Tigrosa, they accounted for the majority of the Araneae collected at that site and had the highest mean MeHg concentrations (Figure 9b). Dolomedes can reach larger sizes than either of the other genera sampled and feed solely on wetland-derived prey, thus these characteristics could be a driving factor contributing to the elevated MeHg concentrations (Bleckmann & Lotz, 1987; Yang et al., 2016).

Figure 9. Spider MeHg Concentrations and Trophic Level



Note: a) Pooled methylmercury concentrations observed in *Agelenopsis*, *Dolomedes*, and *Tigrosa* specimens collected over three consecutive summers from the three study sites: Forest, Wooded Site Wetland (WS), and Open Site Wetland (OS). b) Pooled methylmercury concentrations plotted against $\delta^{15}N$ isotopic values for the three dominant genera present in the study sites. $\delta^{15}N$ was adjusted by site using baseline data from sampled leaf litter and algae at each location. Higher concentrations of MeHg were observed in specimens collected from the wetland sites and genera with higher $\delta^{15}N$ isotopic concentrations were found in at OS. Data are plotted as means from pooled samples.

Differences observed in mean MeHg concentrations between the three sampling locations (Forest, OS, and WS) are likely explained by differences in trophic level, age, size, and other species-specific traits. δ15N was measured for specimens collected and show that the specimens collected at the WS site, Agelenopsis, had lower δ15N isotopic values, while Tigrosa (commonly found at the Forest and OS site) had had higher δ15N isotopic values (Figure 9a-b). Of note, Dolomedes specimens, the dominant genera found at the OS site, were the largest Araneae specimens collected for all three sites (Figure 9a).

A major limitation of this study was the presence of differing Araneae taxa at each site (Forest, OS, and WS). Samples of different species had to be aggregated for analyses introducing a potential bias that must be considered when reviewing the results and figures.

2.4 Conclusion

In this study, we found the two artificial wetlands we studied displayed seasonal variation in MeHg concentrations (with warmer months having higher concentrations in the summer and fall months), and of the two, the wooded site wetland (WS) had notably higher concentrations of THg and MeHg within its sediments for the years in the study period. Sentinel species sampled showed higher concentrations of MeHg at the open site wetland (OS) which contrasts the trend in water and sediment samples collected from this site. Higher biotic MeHg concentrations at OS could be attributed to higher Hg bioavailability as results show that MeHg concentrations normalized to organic matter content, were higher at OS. Mean MeHg concentrations in pooled *Neurocordulia* larva samples were also observed to be significantly higher at the OS wetland compared to WS ($p<0.01$). This pattern can be explained by several factors:

First, previous studies have shown that acidic waters can have the potential to stimulate MeHg production and that invertebrates in acidic systems can take up more MeHg, but pH was not measured during invertebrate sampling for OS and WS though OS is situated near several *Pinus* trees (Clayden et al., 2014; Roy et al., 2009). Second, WS likely has lower MeHg bioavailability due to lower organic matter present in its sediments which was supported by the data provided earlier (normalized MeHg). OS is also characterized by shallow waters, and occasional drying and rewetting periods, which have been shown to stimulate MeHg production (Feng et al., 2014; Oswald & Carey, 2016) and along with its other landscape features (less

dynamic system, potentially longer food chain, etc.) could explain the results of this study (Edmonds et al., 2012, Primm and Lawton, 1977).

Wetlands are uniquely rich ecosystems that can serve as powerful tools for stormwater management in urban areas. Given their ability to produce MeHg, a pollutant of great concern in North Carolina (along with other southeastern states) it is vital to understand Hg cycling in constructed wetlands and ponds known as artificial wetlands. Compounded with the increasing effects of climate change in cities, the potential for urban wetlands to become hotspots for MeHg production like natural wetlands exists and underscores the need to better understand the roles these systems in Hg cycling. Artificial wetlands remain severely understudied although they have been shown to have the potential to methylate Hg. Our results indicate that landscape features play a pivotal role in the production and availability of MeHg artificial wetlands and should thus more consideration to factors influencing MeHg should be included in the construction process.

2.5 Supplemental Information and Figures

2.5.1 Additional site information

Construction was completed in March of 2017, with one wetland created near several recreational fields, in the prairie and the second wetland constructed in a more rural, heavily forested, area of the university. The areas were chosen through a long process considering existing features (such as permeability of the soil, dominant tree species, and site history).

Soil cores taken from OS before construction began revealed this site to be composed mostly of clay, so an aquatic-safe, fish-grade PVC liner surrounded by several geotextile pads was installed to help retain water within the wetland (Figure 10). This site also occurs in a more developed part of the park, with less vegetation and more human activity present.

WS was installed near a naturally occurring inundated spot and was created above a semi-impermeable rock layer (Figure 10).

2.5.2 Details regarding analyses

To prepare for THg analysis water samples were digested using an acidic mixture of KMnO4 and K2S2O8 in an oven at 60^{o}C overnight following Woerndle et al. (2018) and were then ready to be analyzed. Digested samples were cooled and then neutralized with hydroxylamine (30% $NH_2OH.HCl$) and the entire subsample of ~125mL was placed into a clean Hg-free glass bubble for analysis. Samples were analyzed by double amalgamation, a technique using Hg quantification by cold vapor atomic fluorescence spectrometry (CVAFS) via Brooks Rand Model III. For quality control, a calibration curve from 0 to 1ng was developed using a Hg working standard (NIST-3133) and then verified using a prepared secondary standard (NIST-1641d 1ng/mL). Subsequent THg concentrations were reported in ng of Hg per liter (ng/L), with an established method detection limit of 0.10 ng/L. Following the addition of the bubbler, 200uL of 20% tin(II)chloride (SNCL2) was added to reduce Hg(II) to gaseous Hg(0). Then, Hg(0) was purged together by a stream of Hg-free nitrogen gas (N_2) for 15 min to transfer Hg(0) onto a gold trap. The gold trap is then placed onto a Brooks Rand Model III cold vapor atomic fluorescence spectrometer (CVAFS) where Hg(0) was heat-desorbed and measured (USEPA, 2001).

To remove matrix interferences, in MeHg analysis, ~100mL of water subsample was distilled in acid-cleaned 50mL Teflon distillation vials that were subsequently placed in an 125^{o}C aluminum hot block. Samples were kept in the heat block until approximately all of the samples had distilled into a cold Teflon receiving vial (3-4 hours) and then ready for analysis. Distillates were buffered with sodium acetate, CH3COONa, then ethylated by ice-cold 1% sodium tetraethylborate, $NaDEt_4$, for 25 min, with agitation occurring at 5 min intervals in a glass

bubbler. Immediately following, samples were purged with Hg-free N2 gas for 7 minutes to concentrate organic Hg species onto the Tenax TA traps. Traps were then dried using Hg-free N2 gas for an additional 7 minutes before they were transferred to the Brooks Rand Model III CVAFS with an isothermal gas chromatographic separation and pyrolytic unit where MeHg was quantified (Bloom, 1989; Horvat et al., 1993). The MDL or method detection limit for MeHg in water samples was established at 0.04ng/L for a 50mL analyzed sample. Any samples that had concentrations of MeHg below the detection limit were assigned a value of half of the detection limit (ie., 0.02 ng/L) for reporting purposes. To develop the calibration curve of 0 to 0.5 ng, a MeHg calibration standard was used and regularly verified against an in-house THg standard (NIST-3133) via (USEPA, 2001).

For MeHg analysis of biota samples, aliquots of 10-200µL of digested samples were transferred into glass bubblers containing 100mL of nanopure water and 200µL of CHCOONa. KOH was used to neutralize the aliquots before the final reagent, ice-cold 1% $NaB(Et)_4$, was added in order to ethylate the samples. After 25 minutes (with repeated agitation every 5 minutes), quantification of MeHg was performed in the same method described earlier (CVAFS). The standard reference materials (DORM-4 and TORT-2) recovery values fell within the certified range for each batch of samples analyzed.

2.5.3 Tables and Figures

Table 1. Hg Concentrations from other Ponds and Wetlands

Research Group	Location/Site	Size/Age of Wetland	THg (ng/L) – surface water	MeHg (ng/L) – surface water	THg (ng/g) – sediment	MeHg (ng/g) – sediment
Open Wetland Site (OS)	Greensboro, NC, USA	0.2 ha; new	2.39	0.04	40.62	0.24
Wooded Wetland Site (WS)	Greensboro, NC, USA	0.1 ha; new	1.15	0.07	125.04	1.10
Greensboro Pond (Morales, unpublished data)	Greensboro, NC, USA	0.5 ha; mature	1.53	0.39	58.79	0.63
Strickman et al. (2018)	Brampton, Canada	1.5 ha ; new	-	-	4.18-8.18	0.04-0.34
Strickman et al. (2018)	Brampton, Canada	0.5 ha; mature	-	-	21.07-33.53	0.34-1.25
Monson, B. (2007) USEPA	Minnesota	1-2 ha; unknown	2.43	0.34	-	-
Ulus, Y. (2020)	Wilmington, NC, USA	250 ha; natural	8.077	1.131	163.456	15.177

Note: Total-mercury and methylmercury concentrations for several artificial wetlands of comparable size apart from a natural wetland located in North Carolina. Average mercury values are reported for both surface water and sediment samples when available. Wetlands (0-7 years) were listed as new and over 7 years as mature based on Austin et al. 2022 (average age when the pond needs to be dredged).

Table 2. Dominant Vegetation present at each Study Site

Site	Dominant Macrophytes	Dominant Surrounding Vegetation
OS	*Sagittaria latifolia*	*Liquidambar*
	Juncus effusus	*Salix nigra*
	Typha latiflolia	*Pinaceae*
WS	-	*Liquidambar*
	-	*Magnolia grandiflora*
	-	*Morus Rubra*
	-	*Acer negundo*
	-	*Nyssa sylvatica*
	-	*Impatiens capensis*

Note: Table describes the dominant species of macrophytes colonizing the wetlands, as well as vegetation contributing to leaf litter inputs.

Table 3. Study Site Characteristics

Site	Water Presence	Macrophytes	Leaf litter	Coordinates	Groundwater Elevation	Sediment Type
Open Wetland Site (OS)	Most of the year	Present	Absent	36.072175, -79.811985	12 cm	Clay Loam
Wooded Wetland Site (WS)	Always	Absent	Present	36.0732041, -79.8091540	10 cm	Silt Loam

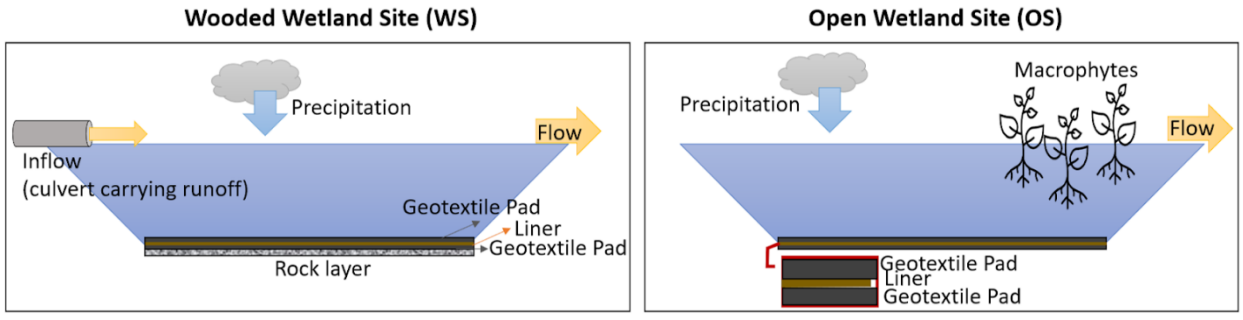
Note: Basic characteristics of each wetland that was created in March 2017 on the University of North Carolina's Greensboro campus.

Table 4. Details on Statistical Analyses

Sample Type	Sample Size and details	Shapiro-Wilk Test Results	Analysis Conducted	Analysis Details	Results (Est, Std. Error, T value, P value)
Water Samples	76, individual monthly samples	Failed: W = 0.69194, p-value = 2.434 e-11	Log transformation then LMER (with dependent variable water MeHg concentration)	Random = Date Factor = Wetland	0.1961, 0.1433, 1.369
Sediment Samples	46, individual monthly samples	Failed: W = 0.9328, p-value =0.01064	Log transformation then LMER (with dependent variable sediment MeHg concentration)	Random = Date Factor = Wetland	1.34839, 0.10455, 12.90
Odonata Samples (biota)	13, pooled samples from same sampling day	Passed: W = 0.89354, p-value = 0.1091	LMER (with dependent variable dragonfly MeHg concentration) One-way ANOVA	Random = Date Factor = Wetland -	-31.912, 8.220, -3.882, 0.00255 ** F-value= 15.07, p-value= 0.00255 **
Aranaea Samples (biota)	10, pooled samples from same sampling day	Passed: W = 0.94928, p-value = 0.66	One-way ANOVA	-	F-value= 0.7088, p-value= 0.5811
Water Sample Seasons	76	-	Log transformation then LMER (with dependent variable water MeHg concentration)	Random = Season Factor = Wetland	0.1864, 0.1750, 1.065
Sediment Sample Seasons	46	-	Log transformation then LMER (with dependent variable sediment MeHg concentration)	Random = Season Factor = Wetland	1.34841, 0.11072, 12.18

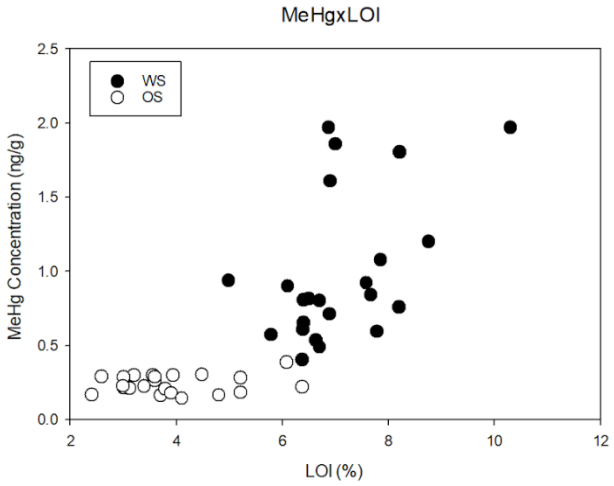
Note: Table provides supplemental details regarding the statistical analyses conducted and the results or R output for each analysis.

Figure 10. Diagram of Study Sites



Note: Basic schematic of water flow patterns and the construction diagram for each study site.

Figure 11. Sediment MeHg concentration and Organic Matter Content



Note: Organic matter content plotted as loss-on-ignition (LOI) with MeHg concentration for OS and WS. %LOI and MeHg concentration for OS are significantly lower ($p<0.05$) than WS.

CHAPTER III: GENERAL DISCUSSION AND CONCLUSIONS

This work was set up to better understand methylmercury production and cycling in two artificial urban wetlands. My research aimed to elucidate the role of artificial urban wetlands in the Hg cycle by understanding the spatial and temporal variation of Hg and investigating the potential transfer of wetland-derived MeHg from the wetland to the riparian food web.

3.1 Questions One and Two

How is methylmercury cycling changing through various seasons and over time in artificial wetlands located in urban areas?

To address these questions, I collected monthly sediment samples (1-3 cm) and surface water samples from two newly constructed wetlands for three years. The two artificial wetlands I studied displayed seasonal variation in MeHg concentrations (with warmer months having higher concentrations in the summer and fall months). There was no notable trend over time, as the wetland sites matured and become established. Water concentrations of THg and MeHg were also not notably different and showed low levels but high variability throughout sampling periods (WS_{MeHg}: 0.06 ± 0.01 ng L^{-1}, OS_{MeHg}: 0.05 ± 0.01 ng L^{-1}; n=38).

Of the two sites, the wooded site wetland (WS) had notably higher concentrations of MeHg within its sediments (WS_{MeHg}: 0.99 ± 0.10 ng g^{-1}, OS_{MeHg}: 0.24 ± 0.02 ng g^{-1}; n=23). I found that MeHg bioavailability was, however, lower for WS, which is consistent with the trends observed in organic matter content for the site and underscores the important role organic material plays in MeHg availability.

3.2 Question Three

Is there evidence of biotransportation of wetland-derived MeHg to terrestrial food webs?

To answer this question, I targeted macroinvertebrates that would emerge from the wetland and riparian Araneae, a common sentinel organism used to elucidate metal pollution (Hannappel et al., 2021; Howie et al., 2018; Yang et al., 2016), focusing my sampling on dominant genera that could transfer MeHg from aquatic to terrestrial food webs. For this part of the study, I had three sampling locations: the wetland sites used for my previous work (3.1) and a Forest site located away from campus streams and wetlands (by approximately 150 m).

I found that sentinel species sampled at the open site wetland (OS) showed higher concentrations of MeHg, contrasting the trend in water and sediment samples collected from this site. Mean MeHg concentrations for Araneae between the three sampling locations (Forest, OS, and WS) show slightly higher concentrations at both wetland sites, and mean methylmercury concentrations in pooled Neurocordulia larva samples were significantly higher at the OS wetland compared to WS ($p<0.01$, $n=7$). Higher biotic MeHg concentrations at OS could be attributed to higher Hg bioavailability as results show that methylmercury concentrations normalized to organic matter content, were higher at OS (based on findings in section 3.1).

3.3 Conclusion and future directions

I found that following the installation of two wetlands MeHg concentrations at these two sites have increased, using campus streams to infer baseline conditions. Concentrations of THg and MeHg in subsurface sediment samples taken at the wooded wetland site (WS) were 3-4x higher than those observed at the open site wetland (OS). My findings indicate certain landscape features (such as a tree canopy which can reduce sunlight and MeHg photodegradation) are

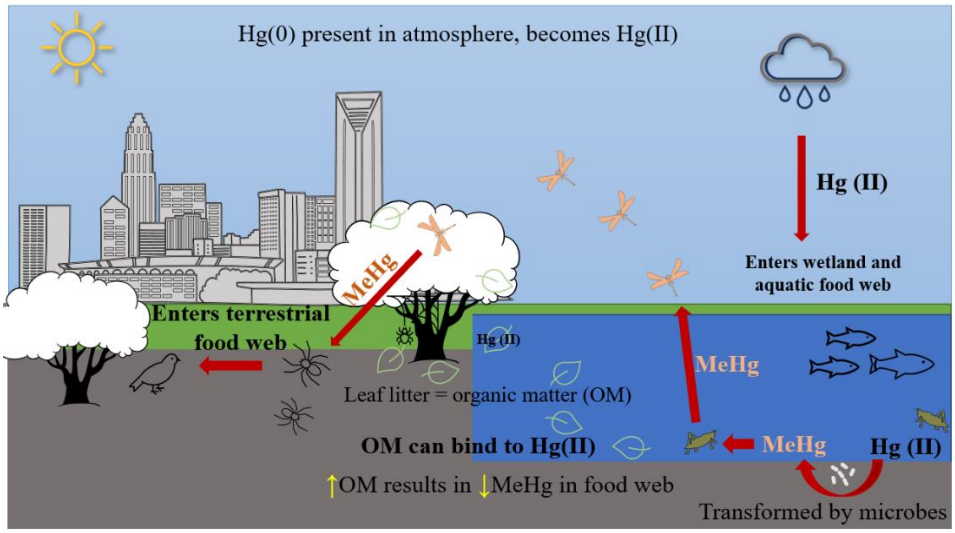
stimulating the production and accumulation of MeHg, given that other factors such as size and age remain the same for both wetlands. Construction practices and the locations where wetlands are installed can contribute to the potential for that wetland to produce elevated concentrations MeHg. Additionally, the biota I sampled had significantly higher MeHg concentrations at OS, further supporting the pivotal role of organic matter in MeHg availability (as this site contained less MeHg in its sediments). A study by Chumchal and Drenner (2015) showed higher MeHg concentrations in emergent aquatic insects from fishless ponds, which can further explain the results I obtained in my study. The OS site lacks the presence of fish, a top predator, in its food web, allowing for a higher aquatic insect diversity and thus more insect-mediated MeHg flux.

Compared to Stickman and Mitchell (the first research group to show in situ MeHg production in artificial urban wetlands) both of my wetland sites had much higher THg concentrations present within their sediment samples (Table 1), and MeHg sediment concentrations that were similar to the highest observed concentrations in their systems; this underscores the importance of evaluating these systems in various climates.

North Carolina (NC) is currently ranked as the fourth fastest-growing state in the United States (Census Bureau, 2021), thus urban areas within the state continue to expand, as does the need for stormwater management solutions, such as artificial wetlands. Due to their unique characteristics, wetlands are versatile tools for stormwater management and pollutant control. Given that MeHg, a pollutant of great concern in NC, is produced in wetlands (Figure 12), coupled with the fact that these systems remain severely understudied, it is vital to understand Hg cycling in constructed wetlands and ponds to inform management and construction practices. The water district staff I interacted with expressed great interest in understanding Hg concentrations and cycling in the systems they managed, and enthusiasm regarding potential

monitoring and mitigation strategies. Organic matter input (via canopy) and food chain length are two potential factors I found that could be altering MeHg bioavailability. My findings help provide a fundamental understanding of how continuing to construct artificial wetlands may enhance MeHg concentrations in urban watersheds and highlights potential factors of concern for future construction practices.

Figure 12. Conceptual Model



Note: Conceptual model of the movement of Hg in an urban artificial wetland.

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Table A5. Data from analyses conducted on sediment samples collected from both wetland sites: Wooded Site wetland (WS) and Open Site wetland (OS). LOI% = loss on ignition; THg=total-mercury; MeHg=methylmercury; MeHg/LOI= MeHg concentration normalized to %LOI; %MeHg=percent of Hg as MeHg.

Site	Sampling Date	LOI %	THg (ng/g)	MeHg (ng/g)	MeHg/LOI (ng:%)	%MeHg
WS	7/11/2018	7.67	167.564	0.842	21.968	0.50
WS	10/31/2018	8.2	125.918	0.759	18.512	0.60
WS	11/28/2018	7.58	124.154	0.922	24.324	0.74
WS	2/19/2019	7.85	108.145	1.078	27.479	1.00
WS	3/26/2019	7	162.02	1.858	53.086	1.15
WS	4/18/2019	10.3	127.814	1.969	38.241	1.54
WS	5/23/2019	8.21	134.141	1.802	43.893	1.34
WS	6/26/2019	6.9	190.314	1.609	46.625	0.85
WS	7/25/2019	6.7	112.11	0.8	23.869	0.71
WS	8/28/2019	6.87	133.81	1.968	57.278	1.47
WS	9/30/2019	8.76	124.0821	1.2	27.386	0.97
WS	10/31/2019	6.1	90.246	0.9	29.495	1.00
WS	11/25/2019	4.98	106.739	0.936	37.59	0.88
WS	12/30/2019	6.4	138.9537	0.652	20.369	0.47
WS	2/26/2020	7.78	106.454	0.593	15.26	0.56
WS	3/19/2020	5.79	81.02	0.571	19.715	0.70
WS	4/22/2020	6.63	115.15	0.534	16.12	0.46
WS	5/27/2020	6.7	75.95	0.488	14.559	0.64
WS	6/17/2020	6.4	112.67	0.807	25.234	0.72
WS	7/29/2020	6.5	101.38	0.814	25.057	0.80
WS	9/23/2020	6.89	98.3616	0.711	20.637	0.72
WS	10/28/2020	6.39	116.616	0.606	18.985	0.52
WS	12/21/2020	6.37	68.0399	0.405	12.705	0.60
Average ± Std. Error	-	**7.09 ± 0.23**	**118.33 ± 6.0**	**0.9923 ± 0.10**	**27.76 ± 2.60**	**0.82 ± 0.06**
OS	7/11/2018	3.11	40.029	0.211	135.536	0.53
OS	10/31/2018	3.56	33.735	0.299	167.978	0.89
OS	11/28/2018	3.94	39.916	0.297	150.585	0.74
OS	2/19/2019	3.59	33.923	0.261	145.216	0.77
OS	3/26/2019	3	26.292	0.215	143.333	0.82
OS	4/18/2019	3.7	26.817	0.161	86.978	0.60
OS	5/23/2019	3.12	33.404	0.209	134.022	0.63
OS	6/26/2019	4.48	46.778	0.304	135.572	0.65
OS	7/25/2019	2.59	42.148	0.2893	222.984	0.69
OS	8/28/2019	3.39	36.875	0.223	131.439	0.60
OS	9/30/2019	6.08	44.226	0.3849	126.519	0.87

OS	10/31/2019	3.2	40.891	0.2968	185.5	0.73
OS	11/25/2019	3	35.675	0.2847	189.8	0.80
OS	12/30/2019	3.59	40.014	0.2876	160.097	0.72
OS	2/26/2020	2.4	40.071	0.1679	139.637	0.42
OS	3/19/2020	2.99	35.843	0.2262	151.102	0.63
OS	4/22/2020	5.21	45.95	0.2804	107.649	0.61
OS	5/27/2020	3.79	38	0.2075	109.473	0.55
OS	6/17/2020	3.9	49.73	0.1774	90.999	0.36
OS	7/29/2020	4.1	33.6392	0.1429	69.736	0.42
OS	9/23/2020	6.37	41.3735	0.2203	69.126	0.53
OS	10/28/2020	5.21	46.0077	0.1819	69.822	0.40
OS	12/21/2020	4.8	37.0571	0.1645	68.549	0.44
Average ± Std. Error	-	**3.87 ± 0.22**	**38.63 ± 1.2**	**0.2388 ± 0.01**	**130.07 ± 8.68**	**0.63 ± 0.03**

Table A6. Data from analyses conducted on water samples collected from both wetland sites: Wooded Site wetland (WS) and Open Site wetland (OS). THg=total-mercury; MeHg=methylmercury; %MeHg=percent of Hg as MeHg; DOC = dissolved organic carbon; DO= dissolved oxygen; Temp. = subsurface water temperature. ND = not determined.

Site	Sampling Date	THg (ng/g)	MeHg (ng/g)	%MeHg	DOC (mg C/L)	DO (mg/L)	Temp. (°C)
WS	10/31/2017	2.22	0.02	0.901	ND	ND	13.4
WS	11/13/2017	2.931	0.02	0.682	3.2	ND	14.1
WS	12/1/2017	1.949	0.106	5.439	6.1	ND	9.5
WS	12/14/2017	2.313	0.077	3.329	ND	ND	5.3
WS	1/25/2018	2.114	0.02	0.946	ND	ND	4.7
WS	2/15/2018	3.729	0.02	0.536	4.6	9.32	9.5
WS	3/5/2018	2.39	0.02	0.837	ND	4.49	9
WS	3/28/2018	3.498	0.02	0.572	3.7	12.6	9.6
WS	4/25/2018	9.352	0.02	0.214	8.8	5.24	13.1
WS	5/23/2018	2.682	0.02	0.746	5.56	0.13	21.5
WS	6/13/2018	2.052	0.081	3.947	9.4	0.1	20.1
WS	7/18/2018	2.863	0.02	0.699	7.91	0.15	24.7
WS	8/30/2018	3.894	0.047	1.207	11.07	1.23	23.7
WS	9/24/2018	3.064	0.292	9.53	16.78	0.27	19.7
WS	10/31/2018	5.596	0.155	2.77	5.44	0.89	9.8
WS	11/28/2018	8.696	0.13	1.495	20.86	2.46	4
WS	12/29/2018	9.023	0.096	1.064	8.57	2.76	7.4
WS	1/29/2019	4.536	0.02	0.441	20.94	5.2	6.4
WS	2/19/2019	5.92	0.02	0.338	5.24	10.6	7.68
WS	3/26/2019	1.924	0.1027	5.338	5.96	10.5	11.2

	WS	4/18/2019	1.714	0.0501	2.923	1.62	0.49	19.6
	WS	5/23/2019	2.996	0.0645	2.153	6.2	3.49	23.2
	WS	6/26/2019	3.614	0.0586	1.621	4.51	0.56	25.8
	WS	7/25/2019	3.406	0.02	0.587	1.17	1.46	21.3
	WS	8/28/2019	5.362	0.0484	0.903	3.9	1.59	22.2
	WS	9/30/2019	5.4787	0.0521	0.951	2.15	2.76	19
	WS	10/31/2019	3.8262	0.02	0.523	4.9	1.74	6.5
	WS	11/25/2019	4.9014	0.0842	1.718	1.75	4.42	14.4
	WS	12/30/2019	3.6466	0.1241	3.403	1.28	13.04	7.6
	WS	2/3/2020	ND	0.02	ND	1.69	11.2	9.7
	WS	2/26/2020	2.45	0.0574	2.339	ND	6.23	14.5
	WS	3/19/2020	2.6551	0.0694	2.614	6.41	0.8	14.2
	WS	4/22/2020	4.0237	0.1506	3.743	ND	3.16	18
	WS	5/27/2020	2.1164	0.0707	3.341	8.87	1.74	16.1
	WS	6/17/2020	3.0627	0.0952	3.108	ND	4.79	24.3
	WS	7/22/2020	4.0904	0.02	0.489	ND	3.98	24.5
	WS	9/23/2020	1.433301	0.02	1.395	ND	4.94	15.4
	WS	10/28/2020	2.5594	0.02	0.781	ND	2.76	16.1
Average ± Std. Error		**-**	**3.73 ± 0.32**	**0.062 ± 0.01**	**1.99 ± 0.31**	**6.73 ± 0.85**	**4.09 ± 0.62**	**14.65 ± 1.06**
	OS	10/31/2017	2.409	0.02	0.83	ND	ND	13.2
	OS	11/13/2017	2.819	0.02	0.709	9.8	ND	15.8
	OS	12/1/2017	1.642	0.013	0.792	9.3	ND	12.5
	OS	12/14/2017	1.639	0.02	1.22	ND	ND	6.5
	OS	1/25/2018	3.574	0.02	0.56	ND	ND	7.4
	OS	2/15/2018	4.655	0.02	0.43	6.9	10.24	14.6
	OS	3/5/2018	2.712	0.046	1.696	8	9.9	10.19
	OS	3/28/2018	3.585	0.041	1.144	6.8	14.41	17.2
	OS	4/25/2018	6.675	0.067	1.004	9.3	8.15	15.3
	OS	5/23/2018	2.664	0.02	0.751	6.82	9.12	26
	OS	6/13/2018	0.904	0.02	2.212	12.7	6.53	23
	OS	7/18/2018	1.138	0.02	1.757	16.09	0.82	26.3
	OS	8/30/2018	5.036	0.02	0.397	19.39	1.9	25.3
	OS	9/24/2018	5.753	0.138	2.399	15.37	2.27	21.3
	OS	10/31/2018	5.787	0.086	1.486	5.7	6.92	10.3
	OS	11/28/2018	9.343	0.098	1.049	17.48	9.91	4.4
	OS	12/29/2018	12.113	0.02	0.165	13.94	10.01	9.3
	OS	1/29/2019	7.565	0.0597	0.789	22.8	13.31	6.7
	OS	2/19/2019	6.298	0.02	0.318	6.33	13.58	7.57
	OS	3/26/2019	4.705	0.1012	2.151	9.59	11.26	12.7
	OS	4/18/2019	6.67	0.3171	4.754	3.95	0.65	21.6
	OS	5/23/2019	2.748	0.02	0.728	14.87	4.52	27.2
	OS	6/26/2019	2.764	0.02	0.724	1.87	2.33	26.5
	OS	7/25/2019	3.427	0.02	0.584	5.57	3.95	23.8
	OS	8/28/2019	5.486	0.1444	2.632	1.88	0.52	23.6

	OS	9/30/2019	2.9107	0.0753	2.587	8.21	2.64	18.1
	OS	10/31/2019	0.0314	0.0469	149.363	9.89	8.74	6.3
	OS	11/25/2019	3.5507	0.02	0.563	1.94	11.89	11.8
	OS	12/30/2019	4.8242	0.02	0.415	2.05	14.12	7.8
	OS	2/3/2020	ND	0.02	ND	2.61	9.4	9.9
	OS	2/26/2020	6.57	0.051	0.776	ND	6.05	17
	OS	3/19/2020	3.2759	0.076	2.32	8.64	6.48	17.3
	OS	4/22/2020	2.9081	0.0891	3.064	ND	4.49	20.8
	OS	5/27/2020	10.1846	0.0802	0.787	12.79	5.04	17.7
	OS	6/17/2020	3.9302	0.0509	1.295	ND	4.64	27
	OS	7/22/2020	2.5635	0.02	0.78	ND	6.41	31.2
	OS	9/23/2020	1.7077	0.02	1.171	ND	9.72	20.9
	OS	10/28/2020	0.8889	0.02	2.25	ND	4.11	17.1
Average ± Std. Error		**-**	**4.20 ± 0.43**	**0.051 ± 0.01**	**5.31 ± 3.95**	**9.33 ± 0.90**	**7.09 ± 0.67**	**16.61 ± 1.18**

Table A7. Data from analyses conducted on water samples collected from creeks near both wetland sites: Downstream of Wooded Site wetland (DWS) and Downstream of Open Site wetland (DOS). MeHg=methylmercury; DOC = dissolved organic carbon; DO= dissolved oxygen; Temp. = subsurface water temperature. ND= not determined.

Site		Sampling Date	MeHg (ng/g)	DOC (mg C/L)	DO (mg/L)	Temp. (°C)
	DWS	10/31/2017	ND	ND	ND	ND
	DWS	11/13/2017	ND	ND	ND	14.30
	DWS	12/1/2017	0.0110	2.00	ND	11.50
	DWS	12/14/2017	0.0000	1.50	ND	8.80
	DWS	1/25/2018	0.0000	2.00	ND	8.00
	DWS	2/15/2018	0.0000	2.50	7.06	14.30
	DWS	3/5/2018	0.0160	ND	9.69	10.90
	DWS	3/28/2018	0.0110	2.80	10.43	15.50
	DWS	4/25/2018	0.0740	6.50	5.62	15.00
	DWS	5/23/2018	0.0350	2.30	2.14	20.40
	DWS	6/13/2018	0.0450	2.50	3.11	20.00
	DWS	7/18/2018	0.0320	3.51	1.45	23.20
	DWS	8/30/2018	0.0100	6.37	5.45	23.70
	DWS	9/24/2018	0.0160	6.02	5.54	20.00
	DWS	10/31/2018	0.0140	6.89	6.74	12.80
	DWS	11/28/2018	0.0080	6.28	8.08	6.60
	DWS	12/29/2018	0.0140	4.44	7.68	12.70

	DWS	1/29/2019	0.0000	3.36	7.75	9.40
	DWS	2/19/2019	0.0167	3.26	10.48	11.06
	DWS	3/26/2019	0.0194	3.15	8.60	12.90
	DWS	4/18/2019	0.0160	0.99	3.72	19.80
	DWS	5/23/2019	0.2929	11.23	2.99	21.30
	DWS	6/26/2019	0.0000	1.00	1.09	23.70
	DWS	7/25/2019	0.0072	0.85	4.63	22.00
	DWS	8/28/2019	0.0203	1.16	0.98	22.50
	DWS	9/30/2019	0.0256	5.02	4.11	20.50
	DWS	10/31/2019	0.0151	1.32	7.02	9.40
	DWS	11/25/2019	0.0145	1.19	7.41	16.20
	DWS	12/30/2019	0.0292	1.08	13.29	11.50
	DWS	2/3/2020	0.0000	1.01	8.16	12.20
	DWS	2/26/2020	0.0000	ND	6.40	15.60
	DWS	3/19/2020	0.0237	3.59	8.36	13.80
	DWS	4/22/2020	0.0000	ND	6.25	18.00
	DWS	5/27/2020	0.0112	2.97	6.41	18.00
	DWS	6/17/2020	0.0111	ND	4.71	24.20
	DWS	7/22/2020	0.0333	ND	4.71	21.20
	DWS	9/23/2020	0.0000	ND	7.98	16.00
	DWS	10/28/2020	0.0152	ND	5.28	16.90
Average ± Std. Error		**-**	**0.023 ± 0.01**	**3.34 ± 0.39**	**6.16 ± 0.46**	**16.05 ± 0.81**
	DOS	10/31/2017	ND	ND	ND	ND
	DOS	11/13/2017	ND	ND	ND	15.70
	DOS	12/1/2017	0.0000	3.80	ND	13.80
	DOS	12/14/2017	0.0040	2.60	ND	8.60
	DOS	1/25/2018	0.0060	3.20	ND	9.30
	DOS	2/15/2018	0.0000	2.60	10.17	14.80
	DOS	3/5/2018	0.0170	ND	9.52	11.30
	DOS	3/28/2018	0.0200	2.80	11.45	15.10
	DOS	4/25/2018	0.0650	10.50	8.55	15.20
	DOS	5/23/2018	0.0210	2.20	7.23	19.50
	DOS	6/13/2018	0.0230	14.10	6.51	19.90
	DOS	7/18/2018	0.0000	3.31	6.28	21.90
	DOS	8/30/2018	0.0190	8.67	5.90	22.80
	DOS	9/24/2018	0.0320	7.48	6.49	20.40
	DOS	10/31/2018	0.0000	11.29	8.14	15.50
	DOS	11/28/2018	0.0130	7.48	7.23	9.20
	DOS	12/29/2018	0.0000	4.69	9.97	13.70
	DOS	1/29/2019	0.0000	1.31	8.83	11.30
	DOS	2/19/2019	0.0000	3.15	12.38	9.89

	DOS	3/26/2019	0.0148	3.27	9.60	13.50
	DOS	4/18/2019	0.0000	1.98	5.61	19.70
	DOS	5/23/2019	0.0770	14.11	5.42	20.80
	DOS	6/26/2019	0.0000	1.87	5.90	22.30
	DOS	7/25/2019	0.0168	2.19	6.02	22.20
	DOS	8/28/2019	0.0046	2.72	5.58	22.20
	DOS	9/30/2019	0.0066	2.92	6.86	20.50
	DOS	10/31/2019	0.0298	3.08	7.62	12.40
	DOS	11/25/2019	0.0000	1.31	7.03	16.70
	DOS	12/30/2019	0.0053	1.59	11.94	14.50
	DOS	2/3/2020	0.0000	0.90	7.90	14.30
	DOS	2/26/2020	0.0000	ND	8.95	15.40
	DOS	3/19/2020	0.0000	2.48	7.73	14.70
	DOS	4/22/2020	0.0000	ND	6.63	18.00
	DOS	5/27/2020	0.0214	4.11	6.66	19.00
	DOS	6/17/2020	0.0212	ND	7.00	23.60
	DOS	7/22/2020	0.0066	ND	7.00	23.60
	DOS	9/23/2020	0.0000	ND	6.20	20.00
	DOS	10/28/2020	0.0131	ND	6.60	18.30
Average ± Std. Error		**-**	**0.012 ± 0.003**	**4.54 ± 0.61**	**7.72 ± 0.30**	**16.75 ± 0.71**

Table A8. Data from analyses conducted on biota samples collected from three sampling locations: Forested area away from both wetlands (Con), Wooded Site wetland (WS), and Open Site wetland (OS). $\delta^{13}C$ = ratio of stable isotopes 13C reported in parts per thousand; $\delta^{15}N$= ratio of stable isotope 15N reported in parts per thousand; Pooled MeHg=methylmercury.

Aquatic Biota					
Site	**Year**	**Genus**	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**	**Pooled MeHg (ng/g)**
OS	2019	Neurocordulia	-28.15	3.72	73.96960182
OS	2019	Neurocordulia	-27.3	4.77	39.29210705
OS	2019	Neurocordulia	-28.62	2.88	27.03344891
OS	2020	Neurocordulia	-29.3	2.97	25.01192733
OS	2021	Neurocordulia	-28.65	2.3	64.53931497
OS	2021	Neurocordulia	-27.08	3.03	51.63883634
WS	2019	Neurocordulia	-34.81	4.87	15.00860615
WS	2019	Neurocordulia	-31.18	4.36	11.19937045
WS	2019	Neurocordulia	-33.58	3.13	11.31136415
WS	2020	Neurocordulia	-34.71	1.88	3.887066718

WS	2021	Neurocordulia	-33.56	3.19	16.60383729
WS	2021	Neurocordulia	-31.8	3.56	16.26470662
WS	2021	Neurocordulia	-27.04	3.55	30.73740258
Terrestrial Biota					
Site	**Year**	**Genus**	**δ13C (‰)**	**δ15N (‰)**	**Pooled MeHg (ng/g)**
Con	2021	Agelenopsis	-24.87	3.63	27.987
Con	2021	Tigrosa	-25.31	7.2	56.37727529
OS	2021	Tigrosa	-26.86	4.51	51.63145198
OS	2021	Dolomedes	-28.43	4.37	77.39354419
OS	2021	Dolomedes	-28.48	5.24	94.63102034
OS	2020	Dolomedes	-28.49	5.45	81.34406935
OS	2020	Tigrosa	-28.08	5.54	33.93409245
WS	2021	Agelenopsis	-31.09	2.87	81.45605001
WS	2019	Agelenopsis	-27.7	2.92	58.45934377