The effect of hydrological restoration on nutrient concentrations, macroinvertebrate communities, and amphibian populations in Lake Erie coastal wetlands

Thesis

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By

Elizabeth Ann Berg

Graduate Program in Environment and Natural Resources

The Ohio State University

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Thesis Committee

Dr. Lauren M. Pintor, Advisor

Dr. S. Mažeika P. Sullivan

Dr. William E. Peterman

Abstract

Historically, coastal wetlands along the western Lake Erie basin supported important ecosystem functions including water quality improvement and biodiversity support. Most remaining coastal wetlands have been diked, severing the hydrologic connection to Lake Erie and its tributaries and acting as barriers to the exchange of water, nutrients, and biota. Concern over harmful algal blooms has led to large-scale coastal wetland restoration initiatives in the western Lake Erie basin. In particular, stakeholders have collaborated to hydrologically reconnect approximately 2,387 acres of protected, diked wetlands in Ottawa National Wildlife Refuge (ONWR). Restoring wetland connectivity has the potential to reduce inputs from the Maumee Area of Concern and reduce amphibian habitat fragmentation. Additionally, wetland restoration is expected to improve macroinvertebrate habitat and food resources in the long-term. However, hydrologic connection to Lake Erie and an impaired watershed may expose biota to new stressors such as nutrient enrichment and invasion of non-native species. In this study, I examined whether hydrologic wetland restoration of coastal wetlands in the western Lake Erie basin had an effect on nutrient concentrations, macroinvertebrate communities, and amphibian populations. Specifically, I compared nutrient concentrations, macroinvertebrate diversity and composition, and amphibian abundance and biomass in 5 restored and 7 diked coastal wetlands. If the reconnection of diked wetlands increased nutrient concentrations, I predicted that biota would experience direct and indirect effects of nutrient enrichment and begin to reflect environmental degradation. Nutrient concentrations varied in restored and diked wetlands, but not always as predicted. Macroinvertebrate communities reflected general properties of ONWR and varied with nutrient concentrations irrespective of the restoration. Amphibian populations were robust to changes in nutrient concentrations and resources. It is likely that large-scale environmental pressures persist and that hydrologic reconnection did not represent the primary limiting factor of macroinvertebrate communities and amphibian populations. Accelerated efforts to protect and restore Lake Erie coastal wetlands are attracting national investment (e.g. Great Lakes Restoration Initiative 2020) as the frequency and intensity of HABs increases. Therefore, it is increasingly important to understand what implications large-scale restoration initiatives have for biodiversity.

Dedication

This work is dedicated to my nephews Daniel and Micah Hayden. May you learn to love and protect our natural resources.

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Vita

University of South Dakota, Vermillion, SD B.S. in Biology, 2016

Publications

Madison, MD, EA Berg, J Alvarado, S Whitfield, A Pinto, and JL Kerby. 2017. Bacterial microbiome isolates from amphibian populations in Costa Rica exhibit varying responses to the fungal pathogen *Bd*. *Frontiers in Microbiology*.

Davis, DR, KJ Ferguson, AD Koch, EA Berg, JR Vlcek, and JL Kerby. 2016. New amphibian and reptile county records from eastern South Dakota, USA. *Herpetological Review*.

Hanlon, SM, D Smith, JL Kerby, EA Berg, MJ Parris, and JE Moore.. 2014. Occurrence of *Bd* in Wapanocca National Wildlife Refuge, Arkansas, USA. *Herpetological Review*.

Field of Study

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1. Introduction

Historically, coastal wetlands along the western Lake Erie basin supported important ecosystem functions including water quality improvement and biodiversity support (Maynard and Wilcox 1997; Zedler and Kercher 2005). Most remaining wetlands have been diked, severing hydrologic connection to Lake Erie and its tributaries and acting as barriers to the exchange of water, nutrients, sediments, and biota. Coastal wetlands, in particular, capture excess nutrients from agricultural runoff and other non-point sources that would otherwise cause eutrophic conditions resulting in harmful algal blooms (HABs) and other undesirable effects. For example, it is estimated Lake Erie coastal wetlands retain 419 t of phosphorus and 5,188 t of nitrogen per year (Sierszen et al 2012). However, these processes are interrupted in diked wetlands. Therefore, large-scale wetland restoration has potential to reduce nutrient input to Lake Erie (Mitsch et al 1989; Wang and Mitsch 1998; Mitsch and Wang 2000). Concern over HABs has led to large-scale wetland restoration initiatives with the underlying goal to improve water quality. In particular, stakeholders have collaborated to hydrologically reconnect approximately 2,397 acres of protected, diked wetlands in Ottawa National Wildlife Refuge (ONWR). Restoring hydrologic connection is expected to reduce nutrient inputs from the Maumee Area of Concern and improve coastal habitat for economically important fisheries and wildlife. However, hydrologic connection to Lake Erie and an impaired watershed may expose biota in previously diked wetlands to new stressors such as nutrient enrichment and invasion of non-native species. Here, I examined whether restoration of coastal wetlands has an effect on nutrient levels and macroinvertebrate diversity in reconnected wetlands in ONWR.

Restoring hydrological connections of coastal wetlands with the surrounding watershed is likely to increase nutrient inputs to the wetlands and alter nutrient concentrations. For example, removal of dikes and water control structures in the wetlands of the Everglades changed this system from its oligotrophic status to being enriched by phosphorus (Surratt et al 2012; Sullivan et al 2014). Similarly, restoration of hydrologic connectivity in the Chesapeake Bay, an impaired watershed characterized by elevated loads of phosphorus, nitrogen, and sediment, led to increased nutrient inputs in reconnected wetlands (Jordan et al 2000; Wolf et al 2013). Early studies at ONWR showed similar effects of hydrologic reconnection on nutrient concentrations. Specifically, Kowalski et al. (2014b) compared water quality in diked and connected wetlands in ONWR. Compared to diked wetlands, connected wetlands had higher levels of soluble reactive phosphorus and nitrate concentrations indicating impaired water quality (Kowalski et al 2014b). Following reconnection of one diked wetland in ONWR, Kowalski et al (2014a) then compared water quality pre- and post-hydrologic reconnection. Post-reconnection, water chemistry began to reflect impaired conditions in Crane Creek and Lake Erie (Kowalski et al 2014a). Specifically, soluble reactive phosphorus increased post-hydrologic reconnection. Given that Crane Creek is a tributary of the Maumee River Area of Concern, an impaired watershed characterized by elevated loads of phosphorus, nitrogen and sediment contributing to HABs in Lake Erie, increased nutrient concentrations in reconnected wetlands may have implications for macroinvertebrate communities in reconnected wetlands.

Nutrients affect macroinvertebrate communities indirectly by altering habitat through changes in wetland vegetation abundance and composition. Added nutrients contribute to excessive emergent vegetation and algal biomass production, reducing open water and submerged vegetation zones and consequently habitat heterogeneity (Maynard and Wilcox 1997). Greater vegetation cover may lead to greater macroinvertebrate abundance and diversity if added habitat meets foraging demands and provides refuge. For example, Heino (2008) examined functional feeding groups and modes of existence of macroinvertebrate communities in lakes related to vegetation cover. He found that herbivore-swimmer, predator-climber, and predator-swimmer abundance were positively related to vegetation cover, likely because vegetation cover provides refuge against fish predation or is related to foraging demands (Heino 2008). However, reduced heterogeneity can have adverse effects. Under conditions of nutrient enrichment, weedy or non-native invasive species such as common reed (*Phragmites australis*) and narrow-leaved cattail (*Typha angustifolia*) may outcompete less aggressive native taxa (Chambers et al 1999; Blann et al 2009). Reed spread and dominance can result in habitat homogenization, which has been associated with reductions in macroinvertebrate diversity and density in wetlands (Raichel et al 2003). In some cases, turnover may at least initially increase habitat heterogeneity, and possibly macroinvertebrate diversity and density. Holomuzki and Klarer (2010) examined common reed dominance and macroinvertebrate diversity and density in southern Lake Erie wetlands. Macroinvertebrate diversity was positively related to reed cover, in part because shading reduced the abundance of floating duckweed that is predominantly colonized by amphipods (Holomuzki and Klarer 2010). Macroinvertebrate diversity and composition may be affected by changes in vegetation if restoring hydrologic connection increases nutrient concentrations in reconnected ONWR wetlands.

Nutrients also structure macroinvertebrate communities by changing food resource abundance and quality. Added nutrients can increase the abundance and diversity of macroinvertebrate communities by increasing primary production and food quality (Rader and Richardson 1994; Heino 2008). Heino (2008) found that macroinvertebrate gathering and shredding functional groups and burrowing mode of existence abundance increased with TP in lakes, likely because fine and course particulate organic matter had higher nutrient content (Heino 2008). However, some macroinvertebrates may only benefit from the addition of nutrients up to the level of enrichment, when there is resource turnover. For example, taxa that rely on periphyton as a food source (i.e. scrapers) would benefit from nutrient inputs up to the level of enrichment that promotes tall emergent plant growth which begins to shade and inhibit periphyton production. Beyond this, taxa that rely on decomposing plant matter (i.e. shredders) may begin to replace periphyton-feeding taxa. King and Richardson (2007) examined macroinvertebrate communities across a phosphorus gradient in Everglades wetlands. They found macroinvertebrate biomass showed a subsidy-stress response to increasing phosphorus enrichment linked to higher quality but lower quantity of periphyton (King and Richardson 2007). Restoring hydrologic connection may indirectly affect macroinvertebrate communities in reconnected ONWR wetlands if added nutrients influence resource abundance and quality.

Finally, increased nutrient concentrations can have indirect effects on macroinvertebrate diversity and composition through changes in water quality. Excessive primary production and decomposition from nutrient addition leads to eutrophication. Many ecological impacts associated with eutrophication result from reductions in dissolved oxygen (DO) (McCormick et al 2004). In general, macroinvertebrate abundance and diversity increase with increasing DO. Rader and Richardson (1994) examined macroinvertebrates in Everglades wetlands and found macroinvertebrate communities shifted from a dominance of Oligochaetes, taxa that are tolerant of low DO, in enriched wetlands to a dominance of sensitive taxa in unenriched wetlands (Rader and Richardson 1994). Reduced DO can also contribute to lower macroinvertebrate densities where adverse changes in water quality exceed the tolerance of most organisms. Therefore, restoring hydrologic connection is expected to restructure macroinvertebrate diversity and composition by modifying local environmental filters, such as nutrient concentrations, which change habitat, food resources, and water quality. Given that nutrients may not always change diversity in predictable ways but rather affect the composition of the community, current research is often focused on understanding how degradation might alter the functional traits and functional diversity of a community (Woodward 2009; Schmera et al 2017).

Multiple macroinvertebrate functional traits have been shown to respond to human-induced environmental changes (Schmera et al 2017). Common functional traits include functional feeding groups, morphological features, modes of existence, life history traits, pH tolerance, and temperature preference. Describing functional traits provides a more detailed interpretation of how environmental stressors affect ecosystem properties. For example, Niemi et al. (2009) examined impacts of urban and agricultural land use on coastal wetland macroinvertebrate functional feeding and modes of existence. They found that the proportion of gatherer-filterers, clingers, and burrowers were associated with inhibited plant growth and elevated turbidity in coastal wetlands with high urban and agricultural land use (Niemi et al 2009). Their findings suggest that urban and agricultural land use altered habitat and water quality, shifting macroinvertebrate community composition towards taxa with traits adapted to altered wetland conditions. In this case, more abundant gatherer-collectors may indicate a shift towards a detrital-based food web (i.e. no sunlight penetrating to fuel algal growth) in wetlands with high urban and agricultural land use. Therefore, describing functional traits in addition to taxonomic diversity provides insight into mechanisms driving changes in macroinvertebrate diversity and composition.

Beginning in 2011, 5 diked wetlands in ONWR were hydrologically reconnected to Lake Erie and Crane Creek. Crane Creek is a tributary of the Maumee River watershed, defined in the Great Lakes Water Quality Agreement as “geographic areas that fail to meet the general or specific objectives of the Agreement where such failure has caused or is likely to cause impairment of beneficial use of the area’s ability to support aquatic life" (International Joint Commission United States and Canada 1987). ONWR offers a unique opportunity to evaluate restoration outcomes of reconnecting diked wetlands to an impaired watershed. Multiple replicates of diked and reconnected wetlands allow for a comparative approach.

I examined the effect of hydrologic reconnection of diked wetlands on nutrient concentrations and macroinvertebrate diversity and community composition. Specifically, my objectives were to 1) quantify and compare phosphorus and nitrogen concentrations in reconnected and diked wetlands, 2) quantify and compare macroinvertebrate diversity and composition in reconnected and diked wetlands, and 3) examine relationships between nutrient concentrations and macroinvertebrate diversity and composition. If the reconnection of coastal wetlands has an effect on nutrient concentrations, then I predicted that 1) nutrients and macroinvertebrates would differ in reconnected and diked wetlands and 2) macroinvertebrate communities would be impaired in wetlands with higher nutrient concentrations.

2. Methods

2.1 Study Site

The ONWR wetland complex is a 2,397-acre freshwater estuarine complex fed by Crane Creek, a small Maumee River tributary. Crane Creek flows into the wetland complex from the west and exits to Lake Erie through a permanent channel between a break in the shoreline dikes on the eastern boundary where Crane Creek and Lake Erie meet to form a freshwater estuary. Wetlands are characterized by tall, emergent vegetation stands (e.g., narrowleaf cattail *Typha angustifolia*) that populate the edges and form stands in the centers of many wetland units, while floating-leaf assemblages (e.g., American lotus *Nelumbo lutea*) extend further from shore. In many wetlands, dense submerged aquatic vegetation mats also extend further form shore. The United States Fish and Wildlife Service manages ONWR via dikes and water control structures and pumps with the primary goals to maximize migratory bird and waterfowl habitat and minimize colonization of invasive plants. Beginning in 2011, five diked units have been hydrologically reconnected to Crane Creek and Lake Erie. We studied the five restored wetlands and eight diked, unrestored wetlands (Figure 1.1).

Unrestored wetlands are hydrologically isolated from Crane Creek and Lake Erie by earthen and rock dikes. Water levels in diked units are artificially managed with occasional drawdowns by pumping creek water in and out depending on management goals. Precipitation, evapotranspiration, and dike overflow also contribute to water level changes in diked units. Unrestored wetlands include MS3, Pool 3, MS4, MS5, MS7A, MS8N, Pool 9E and Pool 1. All unrestored wetlands are adjacent to Crane Creek and Lake Erie and commonly take in water during high water events. In 2011, MS5 water levels were drawn down to maintain migratory bird and waterfowl habitat. MS8B has a possible connection to Crane Creek through a water control structure on the northwestern edge connecting to MS8A, but the structure was not active at the time of this study. Water was exchanged periodically by pumping between MS8B and other wetlands. Pool 1 was restored mid-study in May 2017. Pre-restoration, Pool 1 diked with some water exchange through pumping between Crane Creek and Pool 1.

Restored wetlands are connected directly or indirectly to Crane Creek and Lake Erie by water control structures. ONWR manages water level by occasionally pumping between restored units and Crane Creek, but water levels in restored units are mainly driven by fluctuations in Crane Creek and Lake Erie. Annual water levels in Lake Erie can fluctuate greatly (>1m) depending on antecedent climate. Seasonal water levels can also fluctuate greatly during seichal events. Water control structures allow for water, nutrients, sediment, and biota to exchange between restored wetlands, Crane Creek, and Lake Erie with the exception of some large fishes due to the presence of carp grates. Carp grates are removed after carp spawning season, allowing other large fish to access restored wetlands periodically. Restored wetlands include Pool 2A, Pool 2B, Pool 2C, MS8A, and Pool 1. Pool 2B is adjacent to Crane Creek and has had a continuous open connection since 2011 through a water control structure on the northern edge. Pool 2C is indirectly connected to Crane Creek through a water control structure on the eastern edge of Pool 2C and the east ditch maintains a continuous connection to Crane Creek. Pool 2C had a continuous connection to Crane Creek between 2011-2013. In 2014 and 2015, it was drawn down for construction. In 2017, it was drawn down again for invasive plant management. Pool 2A is adjacent to Crane Creek and has maintained an open continuous connection to Crane Creek indirectly through a water control structure between Pool 2A and Pool 2B. MS8B is adjacent to Crane Creek and has a direct but limited connection through a permanent pump structure on the northwestern edge. The structure was opened periodically (days-weeks at most) for water exchange in 2016 and 2017. Pool 1 is directly connected to Crane Creek by a water control structure on the northwestern edge and has maintained an open continuous connection since May 2017.

2.2 Data Collection

To compare chemical water quality and nutrient levels between restored and unrestored wetlands, I took bi-weekly in situ measurements and water samples for six weeks in 2016 (June—September) and eight weeks in 2017 (May—August). In 2016, I collected 3 water samples per wetland in dominant vegetation zones (emergent, submerged, and floating) (n=72/restored wetlands, n=144/unrestored wetlands). In 2017, I reduced nutrient collection to 1 sample per wetland in open water (n=40/restored wetlands, n=56/unrestored wetlands). Nutrients were collected with acid-washed 250 mL polyethylene bottles, filtered through a 0.45 μm membrane and frozen. Within 3 weeks of collection, samples were transported to the Ohio State University Service Testing and Research Laboratory for Flow Injection Analysis for TN, TP, nitrate-nitrogen (nitrate-N), ammonia-nitrogen (ammonia-N) and phosphate-phosphorus (phosphate-P). At each site where a water sample was collected, I measured temperature (ºC), pH, conductivity (μS/cm), and DO (%) measured on a multi-meter probe (YSI Model 665) and turbidity (NTUs) measured on a handheld spectrophotometer (Hach Turbidimeter).

To compare macroinvertebrate communities between restored and unrestored wetlands, I sampled macroinvertebrates monthly from June-August in 2016 and 2017. I collected 3 samples per wetland in dominant vegetation zones by taking 10 sweep samples in each zone using 500 μm d-frame dipnets (in 2016, n=36/restored wetlands and n=72/unrestored wetlands; in 2017, n=45/restored wetlands and n=63/unrestored wetlands). Macroinvertebrates were subsampled in the field following a method developed by the Great Lakes Coastal Wetland Monitoring plan (Uzarski et al 2017). After 10 sweeps through the water column and vegetation, contents were emptied into white trays with 5 cm grid lines. A total of 150 macroinvertebrates were picked grid by grid with forceps and placed into vials containing 70% ethanol. Picking of macroinvertebrates was limited to 30 person-minutes (e.g. one person picking for 30 minutes, two people picking for 15 minutes). If 150 macroinvertebrates were not picked in 30 person-minutes, then picking proceeded to the next multiple of 50 so that all samples contained 50, 100, or 150 macroinvertebrates (Uzarski et al 2017). Macroinvertebrates were identified to the lowest operational taxonomic unit (OTU) in the laboratory and a subset of samples were sent to Rhithron Associates, Inc. to be identified. Taxonomic keys in Cummins et al. (1996) and Thorp and Covich (2010) were used for identification. Macroinvertebrates where further characterized by four functional trait groups (functional feeding group, modes of existence, body size, and voltinism) based on Cummins et al. (1996), Thorp and Covich (2010), and the Environmental Protection Agency database (Table 1.1).

2.3 Statistical Analysis

To compare nutrient concentrations between restored and unrestored wetlands in 2016 and 2017, I used linear mixed effects models:

$$Nutrient \sim Status + Year + Status * Year + (1|Site)$$

Where “nutrient” represents either TP, TN, or the TN:TP ratio, “status” represents restored or unrestored wetlands, year represents 2016 or 2017, and site is a random effect.

To compare macroinvertebrate diversity and composition between restored and unrestored wetlands in 2016 and 2017, I used linear mixed effects models:

$$Macroinvertebrate\ Metric \sim Status + Year + Status * Year + (1|Site)$$

Where "macroinvertebrate metric" represents one of 24 macroinvertebrate taxonomic and functional metrics, "status" represents restored or unrestored, year represents 2016 or 2017, and site is a random effect. Taxonomic metrics included Shannon diversity (H'), taxonomic richness, Ephemeroptera and Odonata and Trichoptera (EOT) richness, and proportion of dominant taxa. Functional metrics included proportion of each functional trait (Table 1.1), functional diversity, and functional richness. Functional diversity was calculated using Rao's quadratic entropy (RQE), a multidimensional functional diversity index that accounts for species proportions. RQE is measured as the sum of the pairwise distances between species in multidimensional trait space weighted by their proportion (Rao 1982). Functional richness measures the overall spread of traits in a community. Functional richness was measured as the number of unique trait combinations in a community, where higher values indicate more niches are filled by taxa (Villéger et al 2008). In this study, functional richness values were rescaled in the range [0,1] to facilitate interpretation.

To examine whether nutrients were associated with changes in macroinvertebrate diversity and composition within restored and unrestored wetlands in 2016 and 2017, I used linear mixed effects models:

$$Macroinvertebrate\ Metric \sim Status + Nutrient + Nutrient*Year + Status*Year+ (1|Site)$$

Components of the model are described above. Nutrients corresponding to the two weeks closest to when macroinvertebrates were collected were averaged. In some cases, the term *Status*Year* was removed because it did not explain variance in the model.

All statistical analyses were conducted in R version 3.4.2 (R Development Core Team 2017). The lme4 package was used to run linear mixed effects models (Bates et al 2014). P-values were obtained using the lmerTest package (Kuznetsova et al 2017). The marginal (R^2m) and conditional (R^2c) R^2 values were obtained using the r.squaredGLMM function. RQE and functional richness were calculated with the *dbFD* function in the FD package (Laliberté et al 2015). All dependent variables were tested for normality and were transformed if they did not meet normality assumptions. Continuous variables including TN, nitrate-N, ammonia-P, phosphate-P, and functional richness were log-transformed. Additionally, the continuous variable TP was square root transformed. Proportion variables were logit transformed if they were not normal including TN:TP, the proportion of dominant taxa, FC, SC, SH, PI, BU, CB, SK, CN, SW, and bi-multivoltine taxa. The Shapiro-Wilk test was used to test whether transformations were effective. The standard errors of the means were calculated and used to estimate variability of each parameter.

3. Results

3.1 Description of macroinvertebrate assemblage

A total of 10,876 macroinvertebrates in restored and 18,611 macroinvertebrates in unrestored wetlands were collected and identified in 2016 and 2017. I identified 228 OTUs (Appendix A). Hyalellidae (Amphipod) was the most abundant family. Hyalellidae made up 18.59% of restored and 17.57% of unrestored wetland communities (Figure 1.2). Hyalella was the most abundant OTU. Hyalella made up 18.44% of restored and 17.5% of unrestored wetland communities (Figure 1.3).

Eight FFGs were represented: filterer-collector, gatherer-collector, predator, piercer-herbivore, shredder, and scraper. Gatherer-collectors were the most abundant FFG. Gatherer-collectors made up 50.61% of the restored and 50.69% of the unrestored wetland communities (Figure 1.4). Six modes of existence were represented: burrower, climber, clinger, skater, sprawler, and swimmer. Sprawler was the most abundant mode of existence. Sprawlers made up 47.39% of the restored and 43.86% of the unrestored wetland communities (Figure 1.5). Three body size classes were represented: small, medium, and large. Small was the most abundant body size class. Small macroinvertebrates made up 52.47% of the restored and 61.83% of the unrestored wetland communities (Figure 1.6). Three voltinism categories were represented: semivoltine, univoltine, and bi-multivoltine. Univoltine was the most abundant category represented. Univoltine macroinvertebrates made up 72.20% of the restored and 68.74% of the unrestored wetland communities (Figure 1.7).

3.2 The effect of restoration on nutrients

TP was similar between restored and unrestored wetlands (R^2m=0.43, R^2c=0.58, p=0.47) (Table 1.2) (Appendix B, Table B.1). In 2016, TP was significantly higher than in 2017 ($p<0.0001$) (Figure 1.8). There was no significant interaction between status and year (p=0.89). In 2016, mean TP was 0.11 mg/L (± 0.01) and 0.12 mg/L (± 0.01) in restored and unrestored wetlands respectively. In 2017, mean TP was 0.04 mg/L (± 0.01) and 0.05 mg/L (± 0.005) in restored and unrestored wetlands respectively.

TN was significantly lower in restored wetlands than in unrestored wetlands ($R^2m=0.17$, $R^2c=0.36$, p=0.0002) (Table 1.2) (Figure 1.9) (Appendix B, Table B.1). TN was significantly lower in 2016 than 2017 (p=0.0001). However, there was a significant interaction between status and year (p=0.0007) indicating that the difference between years was dependent on status. TN was lower in 2016 than in 2017 within restored wetlands, but was the same between years in unrestored wetlands. Specifically, in restored wetlands mean TN was 0.58 mg/L (± 0.06) in 2016 and 0.99 mg/L (± 0.15) in 2017. In unrestored wetlands, mean TN was 1.36 mg/L (± 0.13) in 2016 and 1.20 mg/L (± 0.1) in 2017.

On average, nitrate-N was similar between restored and unrestored wetlands ($R^2m=0.05$, $R^2c=0.10$, p=0.17) (Table 1.2) (Figure 1.10) (Appendix B, Table B.1). Nitrate-N was similar in 2016 and 2017 (p=0.76). However, there was a significant interaction between status and year (p=0.047). The interaction indicated that the effect of status on nitrate-N depended on year. Specifically, nitrate-N was higher in unrestored wetlands than restored wetlands in 2016, but similar in among wetlands in 2017. It also indicated that nitrate-N was higher in unrestored wetlands in 2016 than in 2017. In 2016, mean nitrate-N was 0.07 mg/L (± 0.01) and 0.29 mg/L (± 0.06) in restored and unrestored wetlands respectively. In 2017, mean nitrate-N was 0.14 mg/L (± 0.06) and 0.14 mg/L (± 0.08) in restored and unrestored wetlands respectively.

Ammonia-N was similar between restored and unrestored wetlands (R^2m=0.24, R^2c=0.27, p=0.45) (Table 1.2) (Figure 1.11) (Appendix B, Table B.1). Ammonia-N was lower in 2016 than 2017 ($p<0.0001$). There was no significant interaction between status and year (p=0.94). In 2016, mean ammonia-N was 0.03 mg/L (± 0.01) and 0.01 mg/L (± 0.003) in restored and unrestored wetlands respectively. In 2017, mean ammonia-N was 0.28 mg/L (± 0.21) and 0.01 mg/L (± 0.01) in restored and unrestored wetlands respectively.

On average, phosphate-P was lower in restored wetlands than unrestored wetlands (R^2m=0.14, R^2c=0.54, p=0.03) (Table 1.2) (Figure 1.12) (Appendix B, Table B.1). Phosphate-P was higher in 2016 than 2017 (p=0.001). There was no significant interaction between status and year (p=0.86). In 2016, mean phosphate-P was 0.04 mg/L (± 0.01) and 0.08 mg/L (± 0.02) in restored and unrestored wetlands respectively. In 2017, mean phosphate-P was 0.04 mg/L (± 0.02) and 0.03 mg/L (± 0.01) in restored and unrestored wetlands respectively.

On average, TN:TP was significantly lower in restored wetlands than unrestored wetlands (R^2m=0.49, R^2c=0.53, p=0.001) (Table 1.2) (Figure 1.13) (Appendix B, Table B.1). TN:TP was significantly higher in 2017 than 2016 ($p<0.0001$). There was a significant interaction between status and year (p=0.01). The interaction indicated that the effect of status on TN:TP depended on year. In 2016, TN:TP was higher in unrestored wetlands but in 2017, TN:TP was similar in restored and unrestored wetlands. In 2016, mean TN:TP was 6.75 (± 1.02) and 12.57 (± 1.19) in restored and unrestored wetlands respectively (Appendix B, Table B.1). In 2017, mean TN:TP was 24.85 (± 2.52) and 29.44 (± 1.98) in restored and unrestored wetlands respectively (Appendix B, Table B.1).

3.3 The effect of restoration on macroinvertebrate communities

The proportion of taxa with small body size was significantly lower in restored than unrestored wetlands ($R^2m=0.12$, $R^2c=0.44$, p=0.04) (Table 1.3) (Figure 1.14) (Appendix B, Table B.2). The proportion of taxa with small body size was similar in 2016 and 2017 (p=0.84). There was no significant interaction between status and year (p=0.53). In 2016, the proportion of taxa with small body size was 52.88% (± 2.74) and 62.33% (± 2.1) in restored and unrestored wetlands respectively. In 2017, the proportion of taxa with small body size was 52.14% (± 3.54) and 61.26% (± 2.14) in restored and unrestored wetlands respectively. The proportion of taxa with medium body size was significantly higher in restored than unrestored wetlands ($R^2m=0.11$, $R^2c=0.29$, p=0.03) (Table 1.3) (Figure 1.15) (Appendix B, Table B.2). The proportion of taxa with medium body size was similar in 2016 and 2017 (p=0.77). There was no significant interaction between status and year (p=0.48). In 2016, the proportion of taxa with medium body size was 29.34% (± 2.27) and 22.26% (± 2.13) in restored and unrestored wetlands respectively. In 2017, the proportion of taxa with medium body size was 28.72% (± 2.52) and 23.32% (± 1.45) in restored and unrestored wetlands respectively.

The proportion of semivoltine taxa was similar in restored and unrestored wetlands ($R^2m=0.07$, $R^2c=0.07$, p=0.14) (Table 1.3) (Appendix B, Table B.2). The proportion of semivoltine taxa was significantly higher in 2016 than 2017 (p=0.02) (Figure 1.16). There was no significant interaction between status and year (p=0.09). In

2016, the proportion of semivoltine taxa was 0.37% (± 0.14) and 0.16% (± 0.1) in restored and unrestored wetlands respectively. In 2017, the proportion of semivoltine taxa was 0.02% (± 0.02) and 0.14% (± 0.07) in restored and unrestored wetlands respectively.

Taxonomic richness, Shannon diversity, EOT richness, and proportion of dominant taxa were similar in restored and unrestored wetlands, similar in 2016 and 2017, and there were no significant interactions between status and year (Table 1.3) (Appendix B, Table B.2). The proportion of all functional feeding group traits including filterer-collectors (FC), gatherer-collectors (GC), piercers (PI), predators (PR), and scrapers (SC) were similar in restored and unrestored wetlands, similar in 2016 and 2017, and there were no significant interactions between status and year (Table 1.3) (Appendix B, Table B.2). Proportions of all modes of existence including burrowers (BU), climbers (CB), clingers (CN), skaters (SK), sprawlers (SP), and swimmers (SW) were similar in restored and unrestored wetlands, similar in 2016 and 2017, and there were no significant interactions between status and year (Table 1.3) (Appendix B, Table B.2). Functional richness and RQE were similar in restored and unrestored wetlands, similar in 2016 and 2017, and there were no significant interactions between status and year (Table 1.3) (Appendix B, Table B.2). The proportion of taxa with large body size was similar in restored and unrestored wetlands, similar in 2016 and 2017, and there was no significant interactions between status and year (Table 1.3) (Appendix B, Table B.2). The proportion of univoltine and bi-multivoline taxa were similar in restored and unrestored wetlands, similar in 2016 and 2017, and there were no significant interactions between status and year (Table 1.3) (Appendix B, Table B.2).

3.4 The effect of nutrient concentrations on macroinvertebrate communities in restored and unrestored wetlands

Taxonomic richness was similar in restored and unrestored wetlands when TP was included in the model (R^2m=0.10, R^2c=0.26, p=0.57) (Table 1.4). Taxonomic richness significantly increased with increasing TP (p=0.01) (Figure 1.17). Taxonomic richness was lower in 2016 than 2017 (p=0.04). There was no significant interaction between TP and year (p=0.07) or status and year (p=0.62). Additionally, taxonomic richness was similar in restored and unrestored wetlands when TN was included in the model (R^2m=0.07, R^2c=0.24, p=0.17) (Table 1.5). Taxonomic richness significantly increased with increasing TN (p=0.047) (Figure 1.18). Taxonomic richness was similar in 2016 and 2017 (p=0.19). However, there was a significant interaction between TN and year (p=0.03) (Figure 1.19). The interaction indicated that the effect of TN on taxonomic richness depended on year. Irrespective of status, taxonomic richness significantly increased with an increase in TN in 2016, and significantly decreased with TN in 2017. There was no significant interaction between status and year (p=0.16).

Shannon diversity was similar in restored and unrestored wetlands when TP was included in the model (R^2m=0.01, R^2c=0.29, p=0.43) (Table 1.4). Shannon diversity significantly increased with increasing TP (p=0.02) (Figure 1.20). Shannon diversity was similar in 2016 and 2017 (p=0.26). There was no significant interaction between TP and year (p=0.32), or restoration status and year (p=0.30). Additionally, Shannon diversity was similar in restored and unrestored wetlands (p=0.14), did not vary significantly with TN (p=0.10), similar in 2016 and 2017 (p=0.08), and there was no significant interaction between TN and year (p=0.15) or status and year (p=0.10) when TN was included in the model (R^2m=0.05, R^2c=0.26) (Table 1.5).

EOT richness was similar in restored and unrestored wetlands (p=0.85), did not vary significantly with TP (p=0.26), similar in 2016 and 2017 (0.17), and there was no significant interaction between TP and year (p=0.08) or status and year (p=0.64) when TP was included in the model (R^2m=0.06, R^2c=0.12) (Table 1.4). Additionally, EOT richness was similar in restored and unrestored wetlands (R^2m=0.11, R^2c=0.23, p=0.41), did not vary significantly with TN (p=0.14), and was similar in 2016 and 2017 (p=0.32) (Table 1.5). There was a significant interaction between TN and year (p=0.01) (Figure 1.21) indicating that the effect of TN on EOT richness depended on year. Irrespective of status, EOT richness did not significantly vary with TN in 2016 but did significantly decrease with an increase in TN in 2017. There was no significant interaction between status and year (p=0.48).

FC was similar in restored and unrestored wetlands when TP was included in the model (R^2m=0.10, R^2c=0.14, p=0.53) (Table 1.4). FC did not vary significantly with TP (p=0.49) and was similar in 2016 and 2017 (p=0.25). There was no significant interaction between TP and year (p=0.14), or restoration status and year (p=0.13). Additionally, FC was similar in restored and unrestored wetlands when TN was included in the model (R^2m=0.13, R^2c=0.15, p=0.06) (Table 1.5). FC significantly increased with increasing TN (p=0.04) (Figure 1.22). FC was similar in 2016 and 2017 (p=0.20). There was no significant interaction between TN and year (p=0.10). However, there was a significant interaction between status and year (p=0.02) (Figure 1.23) indicating that the effect of status on FC depended on year. There were no differences in FC between restored and unrestored wetlands in 2016, but FC was higher in unrestored wetlands in 2017 than in 2016.

GC was similar in restored and unrestored wetlands when TP was included in the model ($R^2m=0.06$, $R^2c=0.25$, p=0.73) (Table 1.4). GC did not vary significantly with TP (p=0.29). GC was similar in 2016 and 2017 (p=0.39). There was no significant interaction between TP and year (p=0.85), or restoration status and year (p=0.39). Additionally, GC was similar in restored and unrestored wetlands when TN was included in the model ($R^2m=0.08$, $R^2c=0.30$, p=0.52) (Table 1.5). GC did not vary significantly with TN (p=0.052). GC was similar in 2016 and 2017 (p=0.92). There was no significant interaction between TN and year (p=0.67) or status and year (p=0.85). However, when the interaction term status*year was removed, GC significantly decreased with increasing TN ($R^2=0.08$, $R^2c=0.30$, p=0.02) (Figure 1.24), but all other relationships were not significant (Table 1.5).

PI was similar in restored and unrestored wetlands when TP was included in the model ($R^2m=0.11$, $R^2c=0.43$, p=0.30) (Table 1.4). PI did not vary significantly with TP (p=0.06). PI was similar in 2016 and 2017 (p=0.22). There was no significant interaction between TP and year (p=0.65), or restoration status and year (p=1.00). Additionally, PI was similar in restored and unrestored wetlands when TN was included in the model ($R^2m=0.08$, $R^2c=0.44$, p=0.94) (Table 1.5). PI significantly increased with increasing TN (p=0.04) (Figure 1.25). PI was similar in 2016 and 2017 (p=0.39). There was no significant interaction between TN and year (p=0.20) or status and year (p=0.44).

SP was similar in restored and unrestored wetlands when TP was included in the model (R^2m=0.12, R^2c=0.19, p=0.17) (Table 1.4). SP did not vary significantly with TP (p=0.051). SP was similar in 2016 and 2017 (p=0.06). There was no significant interaction between TP and year (p=0.56), or restoration status and year (p=0.20). However, when the interaction term status*year was removed, SP significantly decreased with increasing TP (R^2m=0.10, R^2c=0.18, p=0.04) (Figure 1.26), but all other relationships were not significant (Table 1.4). Additionally, SP was similar in restored and unrestored wetlands when TN was included in the model (R^2m=0.12, R^2c=0.25, p=0.86) (Table 1.5). SP did not vary significantly with TN (p=0.08). SP was similar in 2016 and 2017 (p=0.60). There was no significant interaction between TN and year (p=0.85) or status and year (p=0.46). However, when the interaction term status*year was removed, SP significantly decreased with increasing TN (R^2m=0.11, R^2c=0.25, p=0.02) (Figure 1.27), but all other relationships were not significant (Table 1.5).

SW was similar in restored and unrestored wetlands when TP was included in the model (R^2m=0.05, R^2c=0.57, p=0.25) (Table 1.4). SW did not vary significantly with TP (p=0.61). SW was similar in 2016 and 2017 (p=0.66). There was no significant interaction between TP and year (p=0.72), or restoration status and year (p=0.55). Additionally, SW was similar in restored and unrestored wetlands when TN was included in the model (R^2m=0.10, R^2c=0.57, p=0.91) (Table 1.5). SW significantly increased with increasing TN (p=0.02) (Figure 1.28). SW was similar in 2016 and 2017 (p=0.65). There was no significant interaction between TN and year (p=0.43) or status and year (p=0.72).

The proportion of taxa with small body size was similar in restored and unrestored wetlands when TP was included in the model (R^2m=0.14, R^2c=0.43, p=0.07) (Table 1.4). The proportion of taxa with small body size did not vary with TP (p=0.90). The proportion of taxa with small body size was similar in 2016 and 2017 (p=0.48). There was no significant interaction between TP and year (p=0.21), or restoration status and year (p=0.64). Additionally, the proportion of taxa with small body size was similar in restored and unrestored wetlands when TN was included in the model (R^2m=0.15, R^2c=0.47, p=0.07) (Table 1.5). The proportion of taxa with small body size did not vary significantly with TN (p=0.88). The proportion of taxa with small body size was similar in 2016 and 2017 (p=0.995). There was no significant interaction between TN and year (p=0.22) or status and year (p=0.78). However, when the interaction term status*year was removed, the proportion of taxa with small body size was significantly lower in restored wetlands than unrestored wetlands (R^2m=0.15, R^2c=0.47, p=0.04) (Table 1.5), but all other relationships were not significant.

The proportion of taxa with medium body size was similar in restored and unrestored wetlands when TP was included in the model (R^2m=0.15, R^2c=0.30, p=0.06) (Table 1.4). The proportion of taxa with medium body size did not vary significantly with TP (p=0.32). The proportion of taxa with medium body size was similar in 2016 and 2017 (p=0.14). There was no significant interaction between TP and year (p=0.06), or restoration status and year (p=0.59). Additionally, the proportion of taxa with medium body size was significantly higher in restored than unrestored wetlands when TN was included in the model (R^2m=0.15, R^2c=0.30, p=0.04) (Table 1.5). The proportion of taxa with medium body size did not vary significantly with TN (p=0.51). The proportion of taxa with medium body size was similar in 2016 and 2017 (p=0.70). There was no significant interaction between TN and year (p=0.36) or status and year (p=0.50).

The proportion of univoltine taxa was similar in restored and unrestored wetlands when TP was included in the model (R^2m=0.05, R^2c=0.21, p=0.23) (Table 1.4). The proportion of univoltine taxa did not vary significantly with TP (p=0.64). The proportion of univoltine taxa was similar in 2016 and 2017 (p=0.32). There was no significant interaction between TP and year (p=0.93), or restoration status and year (p=0.29). Additionally, the proportion of univoltine taxa was similar in restored and unrestored wetlands when TN was included in the model (R^2m=0.08, R^2c=0.22, p=0.81) (Table 1.5). The proportion of univoltine taxa did not vary significantly with TN (p=0.12). The proportion of univoltine taxa was similar in 2016 and 2017 (p=0.52). There was no significant interaction between TN and year (p=0.52) or status and year (p=0.75). However, when the interaction term status*year was removed, the proportion of univoltine taxa significantly decreased with increasing TN (R^2m=0.08, R^2c=0.22, p=0.047) (Figure 1.29), but all other relationships were not significant (Table 1.5).

Functional richness was similar in restored and unrestored wetlands when TP was included in the model (R^2m=0.12, R^2c=0.12, p=0.75) (Table 1.4). Functional richness significantly decreased with increasing TP (p=0.01) (Figure 1.30). Functional richness was significantly lower in 2016 than 2017 (p=0.01). There was a significant interaction between TP and year (p=0.02) (Figure 1.31). The interaction indicated that the effect of TP on functional richness depended on year. Functional richness decreased with increasing TP in 2016 but did not vary significantly with TP in 2017. There was no significant interaction between restoration status and year (p=0.66). Additionally, functional richness was similar in restored and unrestored wetlands when TN was included in the model (R^2m=0.04, R^2c=0.04, p=0.97) (Table 1.5). Functional richness did not vary significantly with TN (p=0.40). Functional richness was similar in 2016 and 2017 (p=0.94). There was no significant interaction between TN and year (p=0.94) or status and year (p=0.64).

RQE was similar in restored and unrestored wetlands when TP was included in the model (R^2m=0.12, R^2c=0.22, p=0.55) (Table 1.4). RQE did not vary significantly with TP (p=0.17). RQE was similar in 2016 and 2017 (p=0.14). However, there was a significant interaction between TP and year (p=0.03) (Figure 1.32) indicating that the effect of TP on RQE depended on year. RQE decreased with increasing TP in 2016 but increased with increasing TP in 2017. There was no significant interaction between or restoration status and year (p=0.47). Additionally, RQE was similar in restored and unrestored wetlands when TN was included in the model (R^2m=0.10, R^2c=0.15, p=0.11) (Table 1.5). RQE did not vary significantly with TN (p=0.11). RQE was similar in 2016 and 2017 (p=0.73). There was no significant interaction between TN and year (p=0.76) or status and year (p=0.17).

Proportion of dominant taxa was similar in unrestored wetlands, did not vary significantly with TN or TP, similar in 2016 and 2017, and there was no significant interaction between nutrients and year or status and year when either TN or TP were included in the model (Tables 1.4, 1.5). Proportion of functional feeding group traits including PR, SC, and SH were all similar in unrestored wetlands, did not vary significantly with TN or TP, similar in 2016 and 2017, and there was no significant interaction between nutrients and year or status and year when either TN or TP were included in the model (Tables 1.4, 1.5). Proportion of modes of existence including CB, CN, and SK were all similar in unrestored wetlands, did not vary significantly with TN or TP, similar in 2016 and 2017, and there was no significant interaction between nutrients and year or status and year when either TN or TP were included in the model (Tables 1.4, 1.5). Proportion of large body taxa, semivoltine taxa, and bi-multivoltine taxa were all similar in unrestored wetlands, did not vary significantly with TN or TP, similar in 2016 and 2017, and there was no significant interaction between nutrients and year or status and year when either TN or TP were included in the model (Tables 1.4, 1.5).

4. Discussion

4.1 The effect of restoration on nutrients

Overall, nutrient concentration results exhibited some differences in restored and unrestored wetlands, but not as predicted. I predicted that nutrients would be higher in restored wetlands than unrestored wetlands. However, nitrate-N and phosphate-P were lower in restored wetlands than unrestored wetlands in 2016 (Figures 1.10, 1.12), and did not differ in 2017. Additionally, TN was lower in restored wetlands overall (Figure 1.9). These findings are surprising, as they contradict previous studies that showed higher nitrate-N and soluble reactive phosphorus concentrations in restored wetlands compared to unrestored wetlands (Kowalski et al 2014b; Kowalski et al 2014a). However, Kowalski et al. (2014a) observed a decrease in TN following the reconnection of a diked wetland

and attributed this trend to an increase in water volume that may have diluted the concentration of organic nitrogen. Lower nitrate-N, TN, and phosphate-P in restored wetlands in 2016 could reflect an increase in water volume in reconnected wetlands. Additionally, TP, phosphate-P, and ammonia-N were similar in restored and unrestored wetlands in both 2016 and 2017, indicating that hydrologic reconnection had no effect on those nutrients. I measured the dissolved nutrients in the water column, but TP and phosphate-P may have been more abundant in wetland soils. While some phosphorus may be dissolved, it is more commonly adsorbed onto soil particles and removed in wetland systems via sedimentation (Woltemade 2000). Perhaps differences in TP and phosphorus were not detected because phosphorus was sequestered in the soil. It is also possible that wetland management and intense storm events created similar conditions in restored and unrestored wetlands. Water levels in all wetland units are artificially managed with occasional drawdowns by pumping water in and out of the units depending on management goals. In 2017, one of the restored wetland units was drawn down to control for invasive wetland plants. Additionally, intense precipitation and seichal events caused dike overflow in unrestored wetlands in 2017 during the time of this study (Ron Huffman pers comm). Therefore, lower or similar nutrient concentrations in restored wetlands compared to diked wetlands may be an effect of increased water volume, lack of phosphorus in the water column, and wetland management and storm events. To better understand nutrient dynamics in restored wetlands, future studies should focus on measuring nutrient load, nutrient retention, or nutrient mass-balance.

The annual differences in nutrient concentrations observed in restored and unrestored wetlands may be due to annual differences in precipitation and the intensity of storm events. The Ohio EPA reported that water year (wy) 16 was the lowest TP and TN loading year for all western Lake Erie basin watersheds, including the Maumee River watershed, whereas wy15 and wy17 were the highest loading years (Ohio EPA 2018). Additionally, wy16 had less precipitation statewide, while both wy15 and wy17 were wet years. TN and ammonia-N in ONWR wetlands were lower in 2016 than 2017, and thus, followed these patterns in loading and precipitation in the Lake Erie basin watershed. However, phosphorus concentrations showed the opposite trend relative to annual loading estimates in the WLEB and precipitation. TP and phosphate-P were higher in 2016 than 2017. In this case, the timing of rain events may be more important than annual loading. Nutrient levels can spike when higher precipitation events occur in early-spring (Michalak et al 2013). 2016 experienced higher precipitation events in February-March than in 2015 and 2017. Additionally, agricultural practices can exacerbate this phenomenon. Autumn fertilizer application creates conditions for excess phosphorus runoff in early-spring (Michalak et al 2013). Therefore, annual patterns in wetland nutrient concentrations likely varied due to precipitation patterns in the western Lake Erie basin.

Results show that wetlands shifted from N- to P-limited, likely due to annual fluctuations observed in restored and unrestored wetlands, which has implications for the type of algae and plants that may dominate wetlands. In 2016, restored wetlands were N-limited (Figure 1.13) (Redfield et al 1963). In 2017, restored and unrestored wetlands were P-limited (Redfield et al 1963). The observed shift is likely due to the large annual difference in TN and TP concentrations in restored and unrestored wetlands. Nitrogen and phosphorus are important growth-limiting nutrients of algal and wetland plant assemblages (Scott et al 2005). When nitrogen or phosphorus are limiting, it can lead to dense monocultures of wetland plants that uptake nutrients more efficiently, such as *Phragmites* and *Typha* species (Chambers et al 1999). Low N:P in restored wetlands in 2016 are characteristic of waste-water and runoff from disturbed watersheds (Saunders and Kalff 2001). In P-enriched systems like the Maumee AOC, nitrogen limitation can create ideal conditions for HABs (Watson et al 2016). Therefore, N- and P-limitation in wetlands may have created good conditions for wetland plant monocultures and algae that contribute to HABs.

TP concentrations were consistently above target concentrations for the western Lake Erie basin and in 2016, restored and unrestored wetlands were above target concentrations set for Lake Erie tributaries and coastal wetlands, suggesting impaired water quality. The Lake Erie Binational Nutrient Management Plan Work Group published target TP concentrations for the western Lake Erie basin (<0.015 mg/L), its tributaries (<0.032), and coastal wetlands (at least one instance per year < 0.03 mg/L) (Lake Erie Lakewide Management Plan Work Group 2011). TP concentrations in restored and unrestored wetlands were consistently above these target concentrations in 2016. On average, TP concentrations were 0.11 mg/L and 0.12 mg/L in restored and unrestored wetlands respectively (Appendix B, Table B1). TP concentrations were also above target concentrations in 2017. On average, TP concentrations were 0.04 mg/L and

0.05 mg/L in restored and unrestored wetlands respectively. This suggests that coastal wetlands in ONWR may have impaired water quality irrespective of wetland status.

4.2 The effect of restoration and nutrients on macroinvertebrate diversity and composition

Macroinvertebrate diversity and composition were mostly similar in restored and unrestored wetlands, with the exception of the proportion of taxa with small and medium body size. Contrary to my prediction, results indicate that hydrologic reconnection was not a driving factor of macroinvertebrate diversity and composition. Instead, macroinvertebrate diversity and composition reflect general properties of ONWR and vary with nutrient concentrations irrespective of wetland status.

Differences in the proportion of small and medium bodied taxa between restored and unrestored wetlands may suggest that unrestored wetlands are more degraded compared to restored wetlands. Specifically, the proportion of small bodied taxa was lower in restored wetlands than unrestored wetlands, while the proportion of medium bodied taxa was greater in restored wetlands than unrestored wetlands (Figures 1.14, 1.15). Aquatic macroinvertebrate assemblages shift from K-strategist (larger body size) to r-strategists (smaller body size) when ecological conditions are degraded (Díaz et al 2008). K-strategists require stable environments and are governed by density dependent factors such as habitat and food availability. On the other hand, r-strategists are governed by density independent factors, or disturbances, such as low DO levels and nutrient enrichment. In a degraded environment, it is unlikely that habitat and food availability come into play. Therefore, the proportion of large bodied taxa is associated with species that are better competitors but poor at tolerating disturbance, whereas small bodied taxa are associated with species that better tolerate disturbance but are poor competitors (Díaz et al 2008; Ledger et al 2011). When body size is considered, unrestored wetlands appear degraded compared to restored wetlands because of their higher proportion of small and smaller proportion of medium macroinvertebrates. Restored wetlands had lower nutrients in 2016 than unrestored wetlands, which may have released density independent pressures on larger macroinvertebrates. However, macroinvertebrate body size did not vary significantly with nutrients irrespective of wetland status which suggests that other properties of the restoration not measured in this study may have driven differences in small and medium bodied macroinvertebrates.

All other macroinvertebrate diversity and composition measures were similar in restored and unrestored wetlands. Low responses of biotic communities to restoration projects have been widely reported (White et al. 2017). These patterns can occur if the restored property does not represent a primary limiting factor constraining biotic communities (Palmer et al 2010). In this case, hydrologic connection may not be driving macroinvertebrate diversity and composition in ONWR. Low responses of biota may also be observed if large-scale environmental pressures persist (Palmer et al. 2010). As such, nutrient impairment in the Maumee AOC and in the western Lake Erie basin may have limited responses of macroinvertebrate diversity and composition in ONWR. Indeed, macroinvertebrate diversity and composition reflect nutrient concentrations and general properties of ONWR irrespective of wetland status.

Interestingly, the increase in taxonomic richness and diversity and corresponding decrease in functional richness at higher TP concentrations suggests any new species added to the community were functionally redundant and that shifts in functional traits occurred. Taxonomic richness and Shannon diversity increased with TP overall (Figures 1.17, 1.20), while functional richness decreased (Figure 1.30). This indicates that while the number of taxa increased, they were functionally redundant taxa to what was already present. There is also evidence that nutrient concentrations led to shifts in functional groups. Taxonomic richness increased with TN concentrations overall, while functional richness and functional diversity (measured as RQE) remained constant. This relationship can occur if one trait or a combination of traits is replaced by another. Indeed, both functional feeding group and modes of existence proportions shifted with increasing TN concentrations. With higher TN concentrations, the proportion of the functional feeding groups FC and PI increased (Figures 1.22, 1.25), while GC decreased (Figure 1.24). Additionally, the proportion of the mode of existence SW increased with higher TN concentrations (Figure 1.28), while SP decreased (Figure 1.27). Furthermore, when taxonomic and functional indices show inverse responses to environmental stressors, it suggests that distinct functional traits are lost from the community. For example, many taxa identified in restored and unrestored wetlands were characterized by medium, bi-multivoltine, GC-BU trait combination (Appendix A). If these taxa replace species with unique trait combinations, rare suites of traits may be lost from the community. The replacement of species with unique trait combinations by species with traits that are persistent or not lost from the community can lead to homogenization in the functional role of macroinvertebrates. Lower functional diversity can destabilize macroinvertebrate communities in aquatic ecosystems, as fewer traits are available in the species pool to act as buffers against biotic variation (Cadotte 2011). This can result in the reduction of trophic links and loss of ecosystem functions associated with particular species groups. For example, in some wetland habitats, macroinvertebrate detrivores (e.g. SH) play an important role in litter decomposition by converting coarse particulate organic matter into fine particulate organic matter (Batzer 1996). If the SH functional feeding group trait were lost, the rate of litter decomposition may decline, affecting groups that forage on fine particulate organic matter (e.g. GC or FC). Additionally, declines in the diversity of macroinvertebrates that process detritus have been shown to alter nutrient cycling (Truchy et al 2015). Therefore, nutrient concentrations may have led to more functionally redundant macroinvertebrate composition and shifted functional trait abundances in restored and unrestored wetlands.

Results also suggest that macroinvertebrates had subsidy-stress responses to increasing nutrients. Functional diversity decreased with increasing TP in 2016 when concentrations were higher but increased with increasing TP in 2017 when concentrations were lower. Additionally, taxonomic richness increased with TN in 2016 when concentrations were lower but decreased with increasing TN in 2017 when concentrations were higher. Subsidy-stress responses may occur when added nutrients initially contributes to increased quality and quantity of habitat and food resources, but later limit resource availability of certain species (King and Richardson 2007). N- or P-limitation in relative years could have driven these subsidy-stress responses. For example, wetlands were P-limited in 2017 irrespective of restoration status. During that year, functional diversity and taxonomic richness increased with TP addition. However, in the previous year when TP concentrations were high and not P-limited, functional diversity and taxonomic richness decreased with added TP. By examining functional traits, we can better understand what mechanisms drove the effects of nutrient concentrations on macroinvertebrate taxonomic and functional indices.

The proportion of functional feeding groups and their response to nutrient concentrations in ONWR indicate that fine particulate organic matter (FPOM) is an important benthic food resource that is affected by TN addition. GC were the most abundant functional feeding group overall (Figure 1.4). Generally, GC are more abundant in degraded aquatic systems because they are more tolerant to pollution than other functional feeding groups and can readily obtain FPOM. FPOM has been found to be an important driver of macroinvertebrate community composition in Great Lakes coastal wetlands that are protected from intense mixing because organic sediment accumulates easily (Cooper et al 2007). Interestingly, the proportion of FC, a group that also feeds on FPOM, was much lower than GC irrespective of wetland status. GC made up >50% of functional feeding groups, while FC made up <0.5% in restored and unrestored wetlands. The difference in GC and FC abundance reflects FPOM availability and quality in transport (suspended load) relative to that in the benthos (bed load) (Merritt and Cummins 2006). GC obtain FPOM by foraging on small litter fragments, while FC filter FPOM from the water column. The greater proportion of GC indicates that benthic FPOM is an abundant and high-quality resource in restored and unrestored wetlands.

While GC were the most abundant functional feeding group, there is evidence of a subsidy-stress response to increasing nutrient concentrations. With added TN, the distribution of functional feeding groups shifted. FC and PI increased with higher TN concentrations (Figures 1.22, 1.25), while GC decreased (Figure 1.24). Initially, added TN can increase the quality and quantity of bed load FPOM by increasing periphyton productivity. However, excess TN concentrations fuel algal and emergent vegetation growth that suppress periphyton by shading and decrease bed load FPOM quantity (Sierszen et al 2004). The observed shift in GC to FC likely reflects FPOM availability in the bed load versus the suspended load. These findings are consistent with other studies that have shown subsidy-stress responses of GC taxa (King and Richardson 2007). Additionally, higher TN concentrations likely benefited PI because they feed on algae and emergent vegetation. Overall, these results indicate that general properties of restored and unrestored wetlands favor macroinvertebrates that feed on FPOM, but nutrient addition can shift resource availability.

The proportion of modes of existence and their response to nutrient concentrations in ONWR indicate that conditions favor benthic dwelling macroinvertebrates, but that habitat is affected by nutrient addition. Macroinvertebrate taxa considered to be SP were in higher proportion than SK regardless of wetland status, suggesting that wetland habitat at ONWR favors benthic dwelling species. SP made up >40% of modes of existence, while SK made up <5% in restored and unrestored wetlands (Figure 1.5). SP inhabit the surfaces of floating leaves or fine sediments, while skaters inhabit the water surface film. Added nutrients contribute to excessive emergent vegetation and algal biomass production, reducing open water habitat and increasing habitat heterogeneity (Maynard and Wilcox 1997). Therefore, it is possible that nutrient concentrations have indirectly affected SK habitat in restored and unrestored wetlands by reducing water surface area. Additionally, there is evidence that nutrient concentrations shifted modes of existence proportions irrespective of wetland status. The proportion of SP decreased with both TP and TN concentrations, while the proportion of SW, increased with TN concentration (Figures 1.26, 1.27, 1.29). SW are adapted for short periods of swimming between resting locations, usually on submerged portions of aquatic plants. Therefore, increased nutrient concentrations could have increased SW resting locations, providing additional habitat for foraging and refuge.

The shift from high to low proportion of semivoltine macroinvertebrates from 2016 to 2017, the overall distribution of voltinism traits, and response of univoltine macroinvertebrates to nutrient concentrations indicate poor conditions irrespective of wetland status. Voltinism is a trait that describes macroinvertebrate life cycle length and indicates how quickly a particular species could respond to environmental change (Cummins et al 1996). Shorter-lived species are less susceptible to environmental change because they are exposed to stressors for shorter periods of time than species with longer life cycles and prolonged exposure. Therefore, a decrease in the proportion of longer-living semivoltine (life-cycle ≥ 1 year) macroinvertebrates from 2016 to 2017 irrespective of wetland status suggests that environmental conditions worsened in ONWR overall. Additionally, the overall distribution of voltinism traits suggests that conditions in ONWR favor more robust species. The proportion of semivoltine macroinvertebrates was low in ONWR compared to univoltine (1 life-cycle per year) and bi-multivoltine (life cycle < 1 year) macroinvertebrates. Macroinvertebrate communities were made up of >65% of univoltine taxa and >20% of bi-multivoltine taxa, traits that allow taxa to respond more quickly to environmental change (Figure 1.7). While univoltinism was the most abundant trait, there is evidence that variation in TN concentrations led to a shift in the proportion of univoltine macroinvertebrates. The proportion of univoltine macroinvertebrates decreased with increasing TN irrespective of wetland status (Figure 1.29). Tullos et al. (2009) found that univoltine macroinvertebrates were characteristic of undisturbed river channels, whereas multivoltine macroinvertebrates were characteristic of restored river channels in agricultural catchments. Perhaps, univoltine macroinvertebrates are sensitive to TN concentrations in ONWR irrespective of wetland status because of their proximity to an impaired watershed.

The more abundant macroinvertebrate taxa are characterized by a suite of traits that confer resistance or resilience to anthropogenic disturbance, suggesting degraded habitat irrespective of wetland status. For example, many of the more abundant species that were characterized as GC and SP were also bi-multivoltine (Figure 1.2, 1.3) (Appendix A). Abundant taxa included *Hyalella*, *Gammarus*, and Chironomini, which are common in Great Lakes marshes that are protected from wave energy and pelagic mixing because sediment accumulation creates optimal food and habitat conditions (Cooper et al 2007). Similar conditions likely occur in wetlands in ONWR, even restored wetlands are largely surrounded by earthen dikes that protect them from seichal events. Furthermore, *Gammarus* are common in wetland habitats because of their ability to overwinter (Cooper et al 2007). Other abundant taxa including burrowers and mayflies in the genus *Caenis*, have been found to be positively influenced by urban land use and Naididae in the subclass Oligochaeta are able to tolerate low DO levels (Rader and Richardson 1994; Niemi et al 2009; Schock et al 2014). Therefore, more abundant taxa in restored and unrestored wetlands may indicate that conditions in ONWR are degraded.

Limited differences in macroinvertebrate communities between restored and unrestored wetlands could reflect that there was not sufficient time for diversity and composition to change following hydrologic reconnection. Restored wetlands in this study range from 1 month to 5 years post-restoration. Studies show that macroinvertebrates take 5 to 10 years to statistically converge with reference assemblages in restored and created wetlands, and average values never reach absolute reference levels (Moreno-Mateos et al 2012). Meyer and Whiles (2008) compared macroinvertebrate communities in natural sloughs between 5- and 16-years post-restoration. They found that coarse metrics such as total abundance, biomass, and diversity of macroinvertebrates were similar in restored and natural slough wetlands (Meyer and Whiles 2008). Other studies have shown that macroinvertebrate communities take as long as 15- to 25-years to recover in coastal ecosystems (Borja et al 2010). Therefore, it is possible that not enough time has passed since wetlands were hydrologically reconnected to detect a response from macroinvertebrates.

4.4 Conclusion

Hydrologic reconnection had some impact on nutrient concentrations, but not as predicted. I predicted that nutrients would be higher in restored wetlands, but concentrations were either higher in unrestored wetlands or similar irrespective of wetland status likely due to differences in water volume, lack of phosphorus in the water column, and wetland management and storm events. Annual variation in wetland nutrient concentrations can be attributed to precipitation and timing of fertilizer application in the western Lake Erie basin. N- and P-limitations in wetlands may have created good conditions for wetland plant monocultures and algae that contribute to HABs. Overall, nutrient concentrations in coastal wetlands in ONWR may reflect impaired water quality in both restored and unrestored wetlands.

Macroinvertebrate diversity and community composition reflect general properties of ONWR and varied with nutrient concentrations irrespective of wetland status. Macroinvertebrate taxa with small and medium body size varied in restored and unrestored wetlands, but all other metrics were similar in both restored and unrestored wetlands. Overall, nutrient concentrations led to more functionally redundant macroinvertebrate composition and shifted functional trait abundances in restored and unrestored wetlands. There was also evidence that macroinvertebrate communities had subsidy-stress responses to nutrient concentrations irrespective of wetland status. Additionally, the more abundant macroinvertebrate taxa were characterized by a suite of traits that confer resistance or resilience to anthropogenic disturbance, suggesting that wetlands may be degraded irrespective of wetland status.

Low responses of biotic communities following restoration projects may be due to prevailing abiotic constraints (e.g. degraded water quality, modified flow regime). Therefore, it is possible that large-scale environmental pressures persist in western Lake

Erie coastal wetlands and that hydrologic reconnection does not represent the primary limiting factor of macroinvertebrate diversity and composition. Accelerated efforts to protect and restore Lake Erie coastal wetlands are attracting national investment (e.g. Great Lakes Restoration Initiative 2010) as the frequency and intensity of HABs increases. Therefore, it is progressively more important to understand what implications large-scale restoration initiatives have for biodiversity. Future research should focus on understanding nutrient dynamics in diked and reconnected wetlands and continue to examine both taxonomic and functional diversity. Ultimately, future insight into the relationship between nutrient dynamics and biodiversity will help us understand the implications of coastal wetland restoration.

Tables

Table 1. 1 Macroinvertebrate functional groups, traits, and definitions.

Functional Feeding Group	*Definition*
Filterer-collectors	Suspension feeders
Gatherer-collectors	Deposit feeders
Piercers	Herbivores
Predators	Living animal tissue
Scrapers	Periphyton
Shredders	Living and dead plant tissue
Habit	
Burrowers	Inhabit fine sediment
Climbers	Live on plants or detrital debris
Clingers	Attach to wave-swept reaches
Skaters	Dwell on surface
Sprawlers	Inhabit floating leaves or sediment
Swimmers	Cling to submerged objects between swimming
Body Size	
Small	< 9 mm
Medium	9-16 mm
Large	> 16 mm
Voltinism	
Semivoltine	< 1 Life-cycle per year
Univoltine	1 Life-cycle per year
Bi-multivoltine	> 1 Life-cycle per year

Table 1. 2 Linear mixed effects models where nutrients were the dependent variables, status, year, and the interaction term status*year were the fixed effects, and site was the random effect for TP, TN, nitrate-N, ammonia-N, phosphate-P, and TN:TP. Significance codes: * = 0.05.

	Status		**Year**		**Status:Year**			
	T	***P***	**T**	***P***	**T**	***P***	**R^2m**	**R^2c**
TP	-0.73	0.47	**-7.76**	**<0.0001***	0.14	0.89	0.43	0.58
TN	**4.20**	**0.0002***	**3.90**	**0.0001***	**-3.44**	**0.0007***	0.17	0.36
Nitrate-N	1.41	0.17	-0.31	0.76	**-1.99**	**0.047***	0.05	0.10
Ammonia-N	-0.77	0.45	**5.26**	**<0.0001***	0.08	0.94	0.24	0.27
Phosphate-P	**-2.25**	**0.03***	**-3.30**	**0.001***	-0.17	0.86	0.14	0.54
TN:TP	**3.82**	**0.001***	**9.02**	**<0.0001***	**-2.62**	**0.01***	0.49	0.53

Table 1. 3 Linear mixed effects models where taxonomic metrics were the dependent variables, status, year, and the interaction term status*year were the fixed effects, and site was the random effect for macroinvertebrate metrics. Significance codes: * = 0.05.

	Status		**Year**		**Status:Year**			
	T	***P***	**T**	***P***	**T**	***P***	**R^2m**	**R^2c**
Taxonomic richness	-0.41	0.68	-0.14	0.89	0.21	0.84	0.004	0.21
Shannon diversity	-0.75	0.46	-1.04	0.31	0.87	0.39	0.01	0.26
EOT richness	-0.12	0.91	0.09	0.93	-0.51	0.62	0.01	0.11
Dominant taxa	0.89	0.38	0.44	0.66	-0.93	0.36	0.02	0.10
FC	-0.83	0.41	-0.28	0.78	1.67	0.10	0.07	0.11
GC	-0.42	0.68	-0.89	0.38	1.13	0.26	0.01	0.33
PI	0.65	0.52	0.16	0.87	-0.29	0.78	0.01	0.49
PR	0.71	0.48	1.18	0.24	-1.86	0.07	0.06	0.06
SC	-1.77	0.09	-0.87	0.39	0.68	0.50	0.07	0.23
SH	-1.91	0.06	-0.38	0.71	1.33	0.19	0.07	0.07
BU	0.23	0.82	0.74	0.46	-0.85	0.40	0.01	0.01
CB	-1.05	0.30	0.14	0.89	0.09	0.93	0.04	0.29
CN	-0.16	0.87	-1.03	0.31	-0.15	0.88	0.05	0.05
SK	-0.81	0.42	-1.39	0.17	0.37	0.71	0.05	0.05
SP	-1.31	0.20	-1.34	0.19	1.47	0.15	0.03	0.25
SW	0.84	0.41	0.70	0.49	-0.76	0.45	0.01	0.62
Small taxa	**2.16**	**0.04***	0.20	0.84	-0.64	0.53	0.12	0.44
Medium taxa	**-2.24**	**0.03***	-0.30	0.77	0.72	0.48	0.11	0.29
Large taxa	-0.76	0.45	0.20	0.85	-0.18	0.86	0.02	0.16
Semivoltine taxa	-1.50	0.14	**-2.31**	**0.02***	1.73	0.09	0.07	0.07
Univoltine taxa	-1.36	0.18	-1.65	0.10	1.21	0.23	0.04	0.23
Bi-multivoltine taxa	1.26	0.22	1.80	0.08	-1.13	0.26	0.05	0.27
Functional richness	-0.58	0.56	-0.27	0.79	-0.24	0.81	0.02	0.02
RQE	-0.80	0.43	0.38	0.71	0.90	0.37	0.05	0.13

Table 1. 4 Linear mixed effects models where macroinvertebrate metrics were the dependent variables, status, TP, year, and the interaction terms status*year and TP*year were the fixed effects, and site was the random effect. Significance codes: * = 0.05.

	Status		**TP**		**Year2017**		**TP:Year**		**Status:Year**			
	T	***P***	**T**	***P***	**T**	***P***	**T**	***P***	**T**	***P***	**R^2m**	**R^2c**
Taxonomic richness	-0.57	0.57	**2.80**	**0.01***	**2.13**	**0.04***	-1.83	0.07	0.50	0.62	0.10	0.26
Shannon diversity	-0.81	0.43	**2.29**	**0.02***	1.14	0.26	-1.00	0.32	1.05	0.30	0.01	0.29
EOT richness	-0.19	0.85	1.15	0.26	1.38	0.17	-1.77	0.08	-0.47	0.64	0.06	0.12
Dominant taxa	0.90	0.38	-1.34	0.19	-0.67	0.50	0.51	0.61	-0.93	0.36	0.05	0.11
FC	-0.64	0.53	-0.70	0.49	-1.15	0.25	1.51	0.14	1.53	0.13	0.10	0.14
GC	-0.35	0.73	-1.08	0.29	-0.86	0.39	-0.19	0.85	0.87	0.39	0.06	0.25
PI	1.05	0.30	1.88	0.06	1.25	0.22	-0.46	0.65	-0.01	1.00	0.11	0.43
PR	0.67	0.51	0.19	0.85	0.91	0.36	-0.66	0.51	-1.77	0.08	0.07	0.07
SC	-1.68	0.10	0.32	0.75	-0.61	0.54	0.83	0.41	0.60	0.55	0.10	0.24
SH	-1.80	0.08	0.82	0.41	0.43	0.67	-0.47	0.64	1.26	0.21	0.06	0.06
BU	0.27	0.79	0.22	0.83	0.77	0.45	-0.72	0.48	-0.88	0.38	0.03	0.03
CB	-1.04	0.31	0.90	0.37	0.48	0.64	0.10	0.92	0.09	0.93	0.07	0.29
CN	-0.29	0.78	1.14	0.26	0.41	0.68	-0.76	0.45	-0.05	0.96	0.06	0.06
SK	-0.93	0.35	-0.14	0.89	-0.73	0.47	0.07	0.94	0.46	0.64	0.05	0.05
SP	-1.40	0.17	-1.99	0.051	-1.89	0.06	0.59	0.56	1.29	0.20	0.12	0.19
SP*	-0.66	0.52	**-2.06**	**0.04***	-1.58	0.12	0.72	0.48	NA	NA	0.10	0.18
SW	1.18	0.25	0.51	0.61	0.45	0.66	0.36	0.72	-0.61	0.55	0.05	0.57
Small taxa	1.91	0.07	0.13	0.90	0.71	0.48	-1.27	0.21	-0.47	0.64	0.14	0.43
Medium taxa	-1.99	0.06	-1.02	0.31	-1.51	0.14	1.94	0.06	0.54	0.59	0.15	0.30
Large taxa	-0.71	0.48	0.98	0.33	0.73	0.47	-0.36	0.72	-0.16	0.88	0.04	0.16
Semivoltine taxa	-1.37	0.18	-0.37	0.71	-1.21	0.23	-0.04	0.97	1.62	0.11	0.08	0.08
Univoltine taxa	-1.22	0.23	-0.47	0.64	-1.00	0.32	0.09	0.93	1.08	0.29	0.05	0.21
Bi-multivoltine taxa	1.25	0.22	0.46	0.64	1.05	0.30	0.00	1.00	-1.04	0.30	0.06	0.26
Functional richness	-0.32	0.75	**-2.83**	**0.01***	**-2.55**	**0.01***	**2.37**	**0.02***	-0.44	0.66	0.12	0.12
RQE	-0.61	0.55	-1.38	0.17	-1.50	0.14	**2.28**	**0.03***	0.72	0.47	0.12	0.22

For SP*, the interaction term status*year was removed from the model.

Table 1. 5 Linear mixed effects models where macroinvertebrate metrics were the dependent variables, status, TN, year, and the interaction terms status*year and TN*year were the fixed effects, and site was the random effect. Significance codes: * = 0.05.

	Status		**TN**		**Year2017**		**TN:Year**		**Status:Year**			
	T	***P***	**T**	***P***	**T**	***P***	**T**	***P***	**T**	***P***	**R^2m**	**R^2c**
Taxonomic richness	-1.40	0.17	**2.03**	**0.047***	-1.34	0.19	**-2.28**	**0.03***	1.44	0.16	0.07	0.24
Shannon diversity	-1.52	0.14	1.69	0.10	-1.81	0.08	-1.44	0.15	1.67	0.10	0.05	0.26
EOT richness	-0.83	0.41	1.51	0.14	-0.99	0.32	**-2.77**	**0.01***	0.71	0.48	0.11	0.23
Dominant taxa	1.55	0.13	-1.24	0.22	0.92	0.36	0.46	0.65	-1.32	0.19	0.04	0.08
FC	-1.93	0.06	**2.12**	**0.04***	-1.30	0.20	-1.66	0.10	**2.50**	**0.02***	0.13	0.15
GC	0.65	0.52	-1.98	0.052	0.10	0.92	0.43	0.67	0.19	0.85	0.08	0.30
GC*	1.06	0.30	**-2.41**	**0.02***	0.49	0.62	0.59	0.56	NA	NA	0.08	0.30
PI	-0.08	0.94	**2.01**	**0.04***	-0.87	0.39	-1.29	0.20	0.77	0.44	0.08	0.44
PR	0.83	0.41	-0.47	0.64	1.05	0.30	-0.51	0.62	-1.56	0.12	0.07	0.07
SC	-1.45	0.16	-0.13	0.90	-0.39	0.70	1.58	0.12	0.14	0.89	0.12	0.27
SH	-1.38	0.17	-0.25	0.80	-0.37	0.71	-0.66	0.51	1.25	0.22	0.08	0.08
BU	0.43	0.67	-0.42	0.68	0.93	0.36	0.78	0.44	-1.06	0.30	0.02	0.02
CB	-0.90	0.37	-0.07	0.95	0.42	0.67	1.42	0.16	-0.32	0.75	0.08	0.33
CN	0.74	0.46	-1.47	0.15	-0.56	0.58	-0.66	0.52	-0.38	0.70	0.13	0.13
SK	-0.46	0.65	-0.32	0.75	-1.05	0.30	0.17	0.87	0.18	0.86	0.05	0.05
SP	-0.18	0.86	-1.78	0.08	-0.53	0.60	-0.19	0.85	0.75	0.46	0.12	0.25
SP*	0.49	0.63	**-2.47**	**0.02***	0.21	0.83	0.19	0.85	NA	NA	0.11	0.25
SW	0.12	0.91	**2.37**	**0.02***	-0.45	0.65	-0.80	0.43	0.36	0.72	0.10	0.57
Small taxa	1.87	0.07	-0.16	0.88	0.01	1.00	-1.23	0.22	-0.29	0.78	0.15	0.47
Small taxa*	**2.17**	**0.04***	-0.01	0.99	-0.47	0.64	-1.58	0.12	NA	NA	0.15	0.47
Medium taxa	**-2.15**	**0.04***	0.66	0.51	-0.39	0.70	0.92	0.36	0.67	0.50	0.15	0.35
Large taxa	-0.35	0.73	-0.40	0.69	0.39	0.70	0.34	0.74	-0.38	0.71	0.03	0.18
Semivoltine taxa	-1.10	0.28	-0.20	0.84	-1.69	0.10	1.28	0.20	1.11	0.27	0.10	0.10
Univoltine taxa	-0.24	0.81	-1.59	0.12	-0.65	0.52	0.64	0.52	0.32	0.75	0.08	0.22
Univoltine taxa*	-0.02	0.99	**-2.03**	**0.047***	-0.73	0.47	0.91	0.37	NA	NA	0.08	0.22
Bi-multivoltine taxa	0.30	0.76	1.54	0.13	0.81	0.42	-0.72	0.48	-0.27	0.79	0.09	0.26
Functional richness	0.04	0.97	-0.85	0.40	0.07	0.94	0.08	0.94	-0.47	0.64	0.04	0.04
RQE	-1.65	0.11	1.64	0.11	-0.35	0.73	-0.31	0.76	1.39	0.17	0.10	0.15

For GC*, SP*, small taxa*, and univoltine taxa*, the interaction term status*year was removed from the model.

Figures

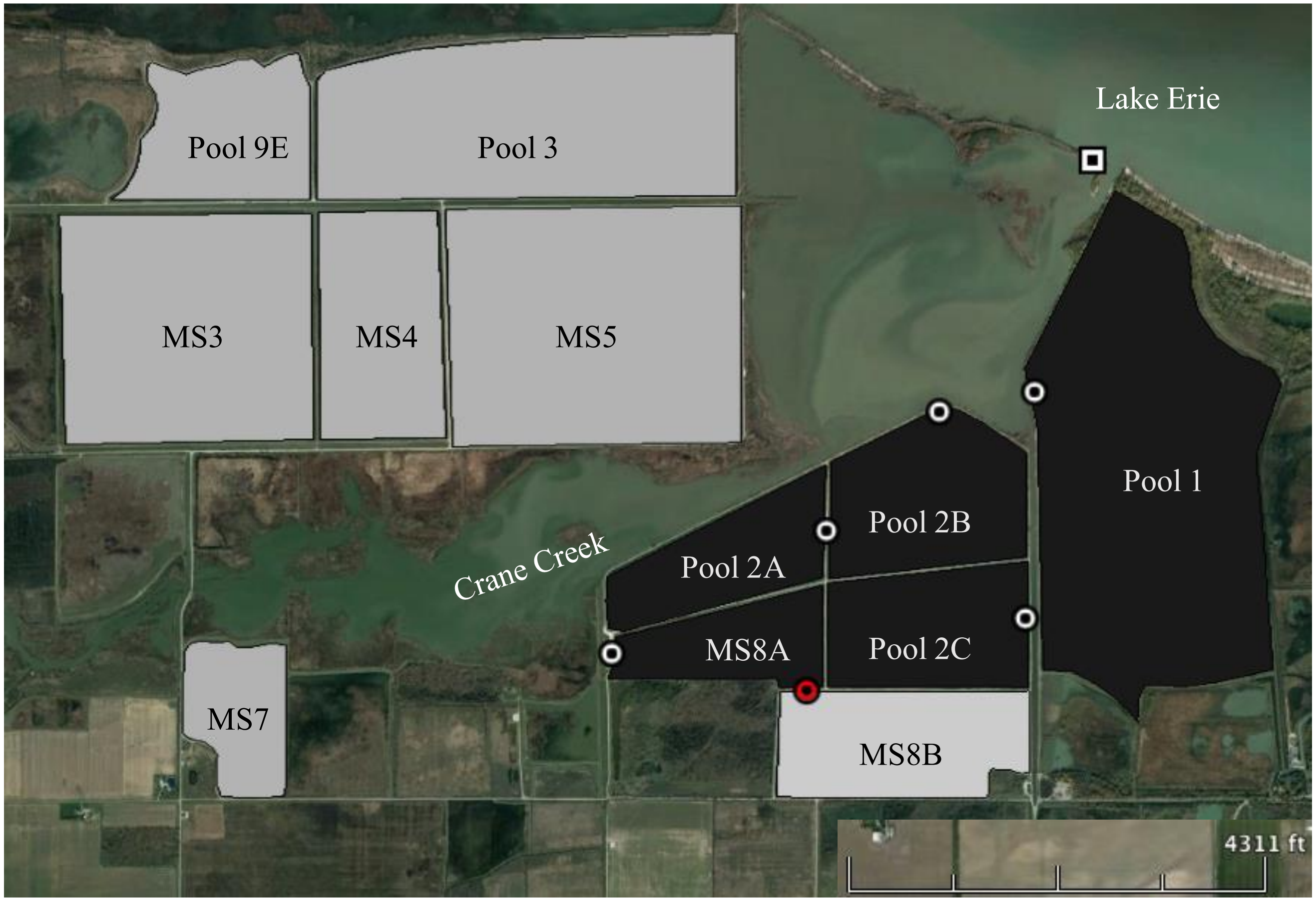

Figure 1. 1 Ottawa National Wildlife Refuge complex. Unrestored wetlands indicated by gray polygons, restored wetlands indicated by black polygons. Active water control structures indicated by white circles, inactive water control structure indicated by red circle. Opening to Lake Erie indicated by white square.

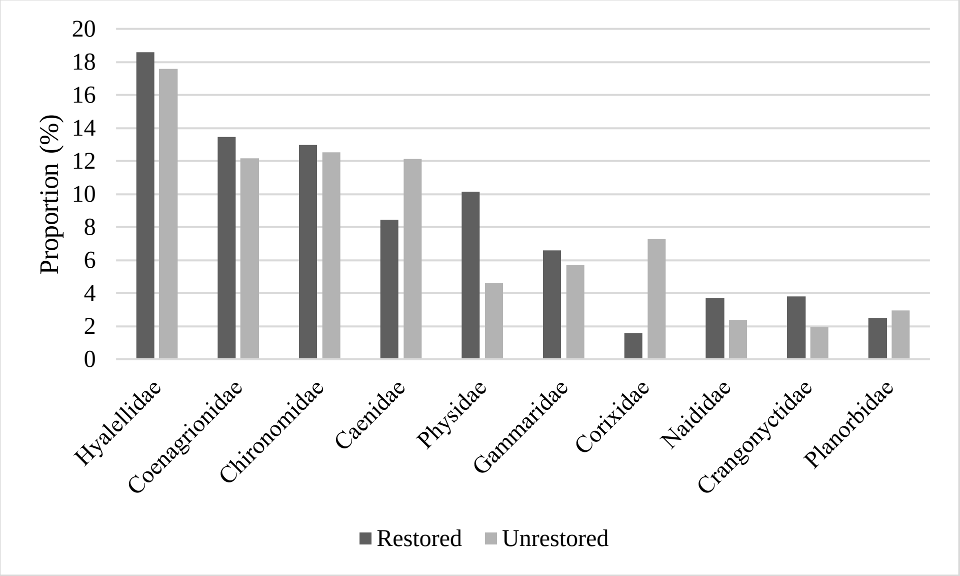

Figure 1. 2 Proportion of overall 10 most abundant families in restored and unrestored wetlands.

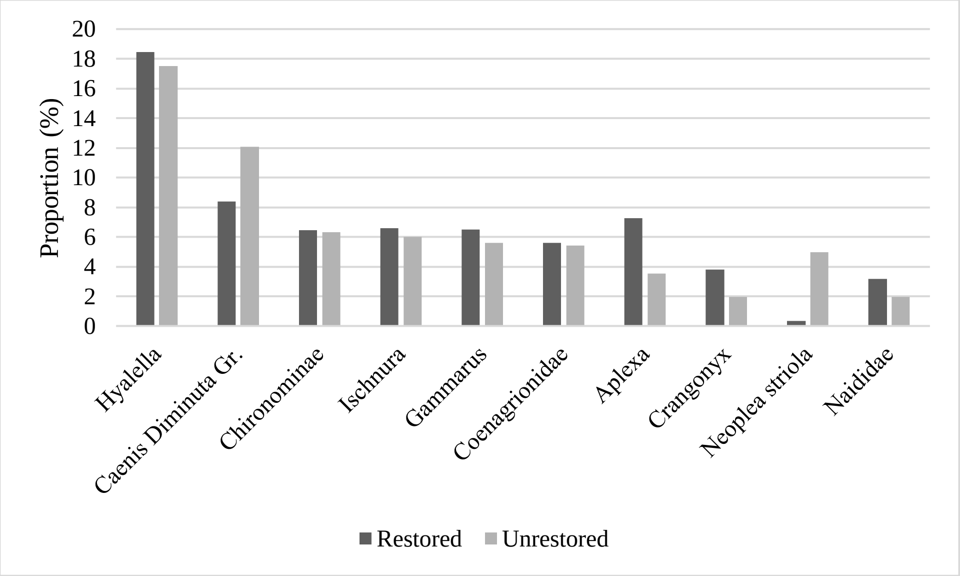

Figure 1. 3 Proportion of overall 10 most abundant families in restored and unrestored wetlands.

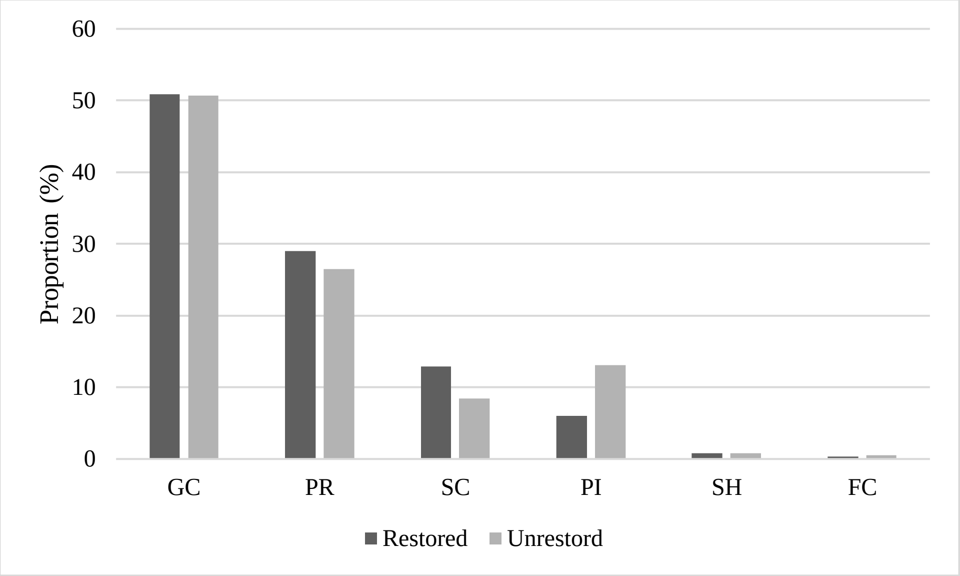

Figure 1. 4 Proportion of FFGs in restored and unrestored wetlands.

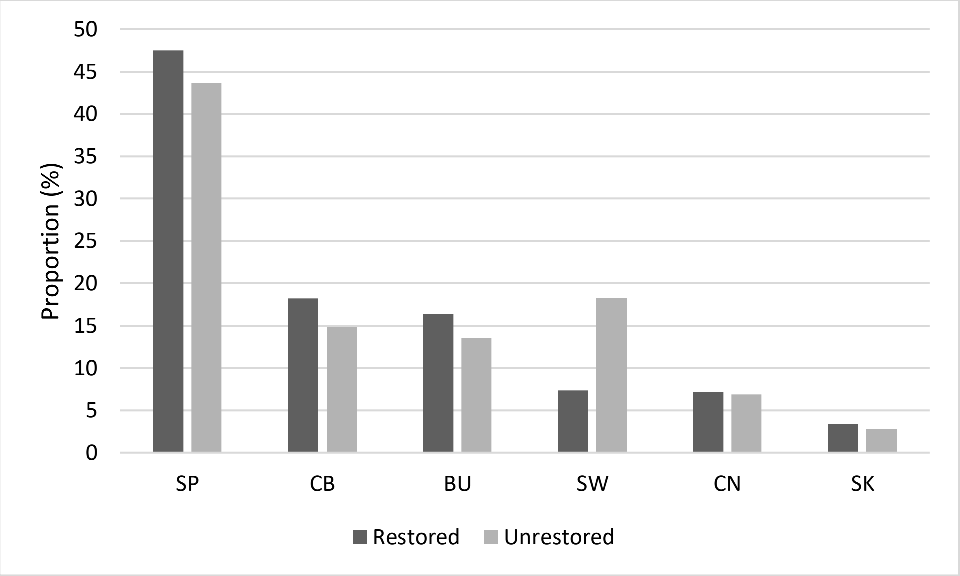

Figure 1. 5 Proportion of modes of existence in restored and unrestored wetlands.

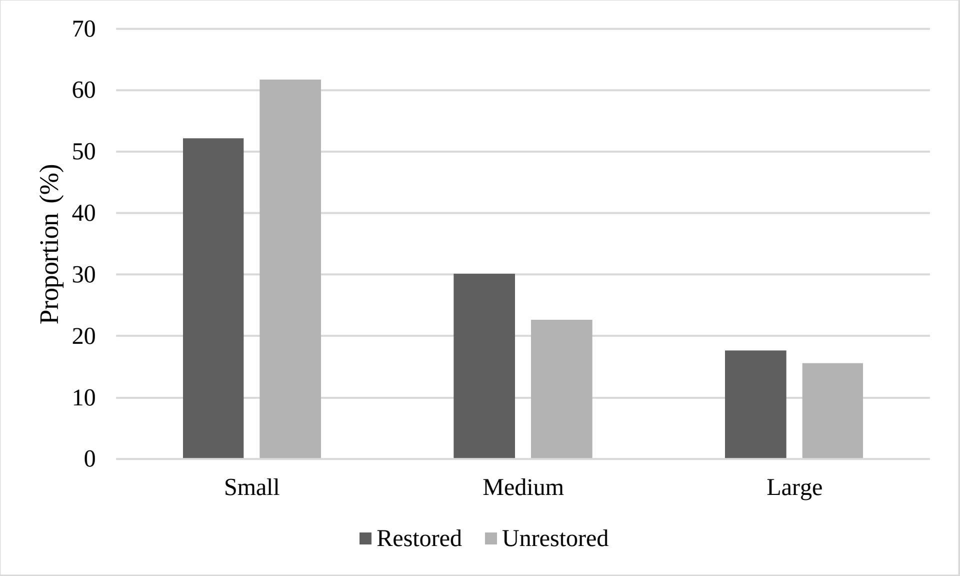

Figure 1. 6 Proportion of body size classes in restored and unrestored wetlands.

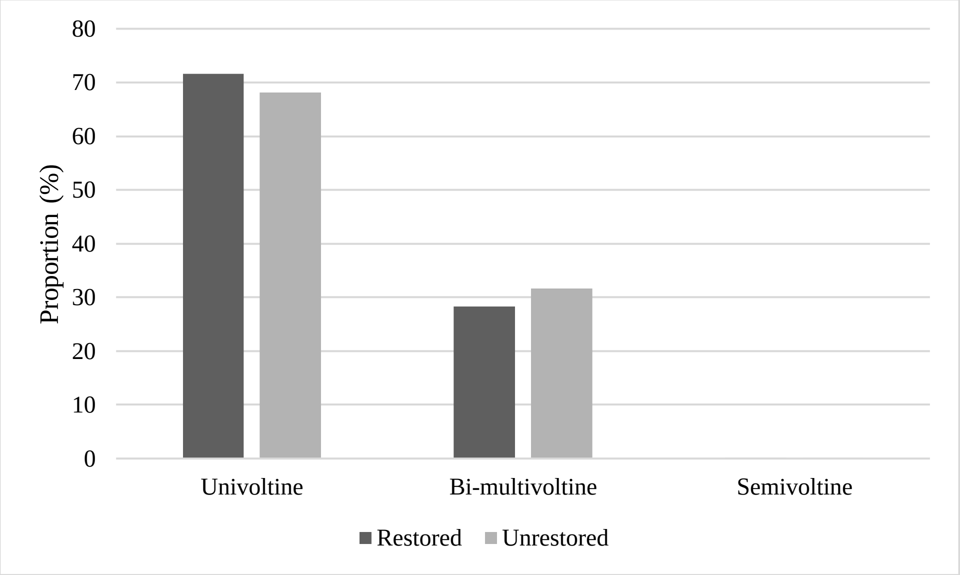

Figure 1. 7 Proportion of voltinism traits in restored and unrestored wetlands.

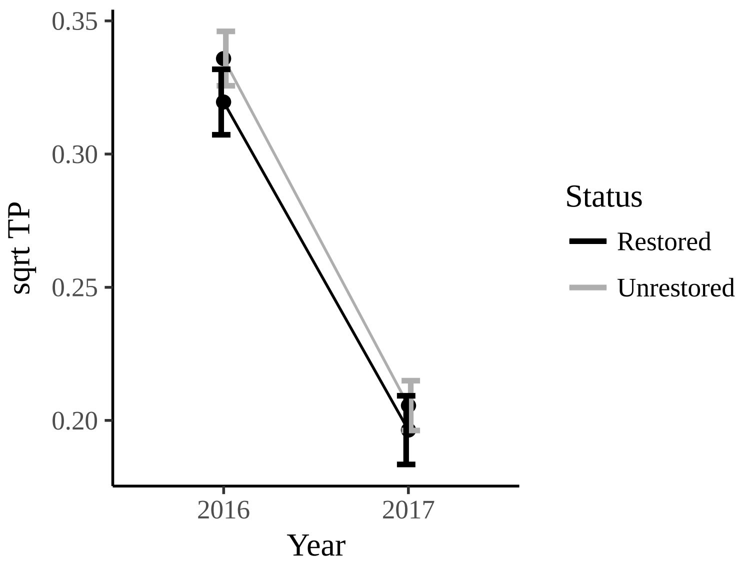

Figure 1. 8 Square root transformed TP in restored and unrestored wetlands in 2016 and 2017.

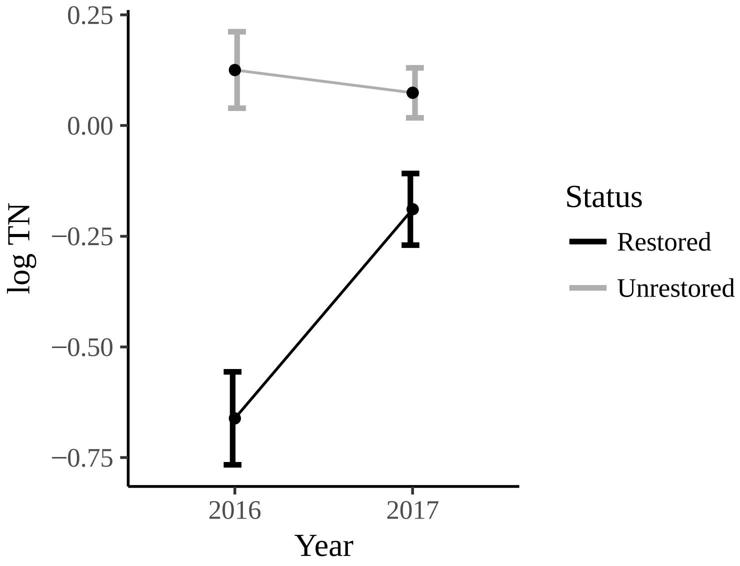

Figure 1. 9 Log transformed TN in restored and unrestored wetlands in 2016 and 2017.

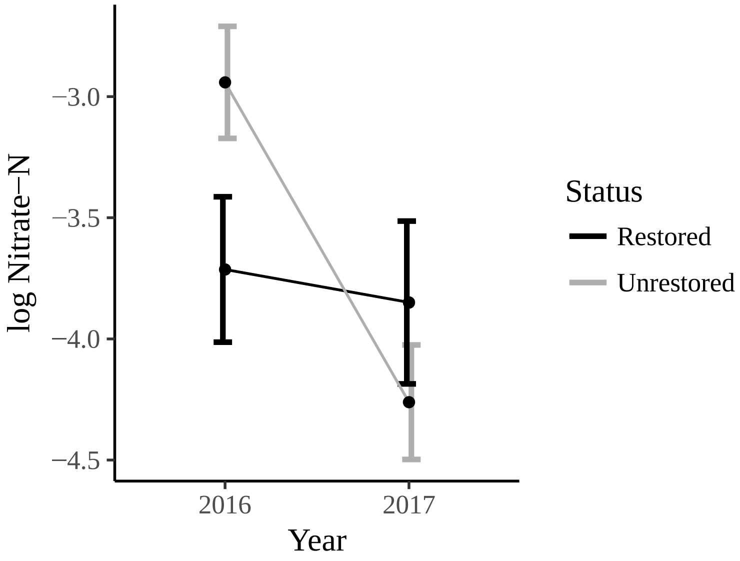

Figure 1. 10 Log transformed nitrate-N in restored and unrestored wetlands in 2016 and 2017.

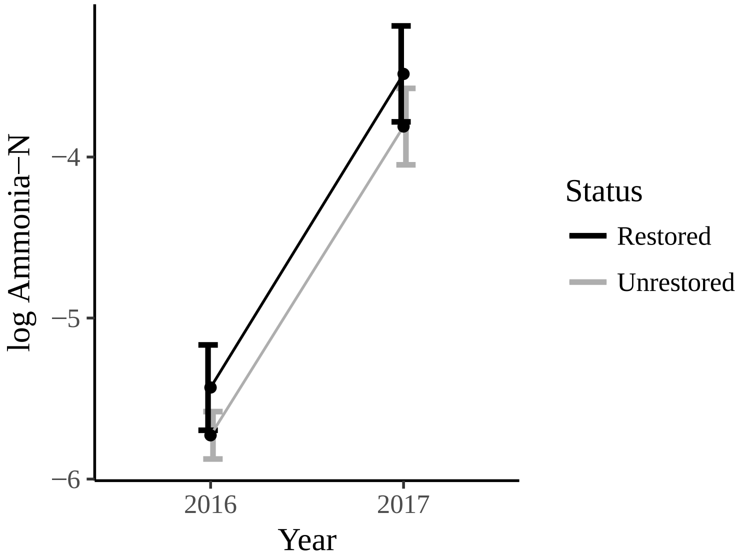

Figure 1. 11 Log transformed ammonia-N in restored and unrestored wetlands in 2016 and 2017.

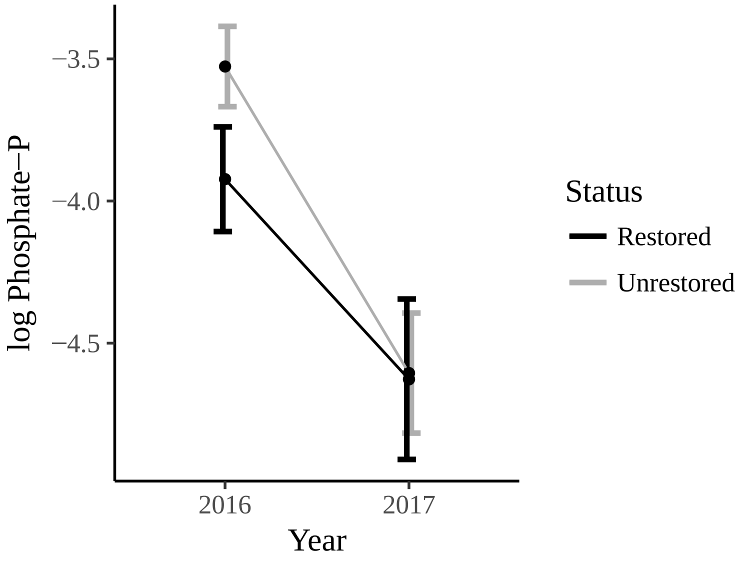

Figure 1. 12 Log transformed phosphate-P in restored and unrestored wetlands in 2016 and 2017.

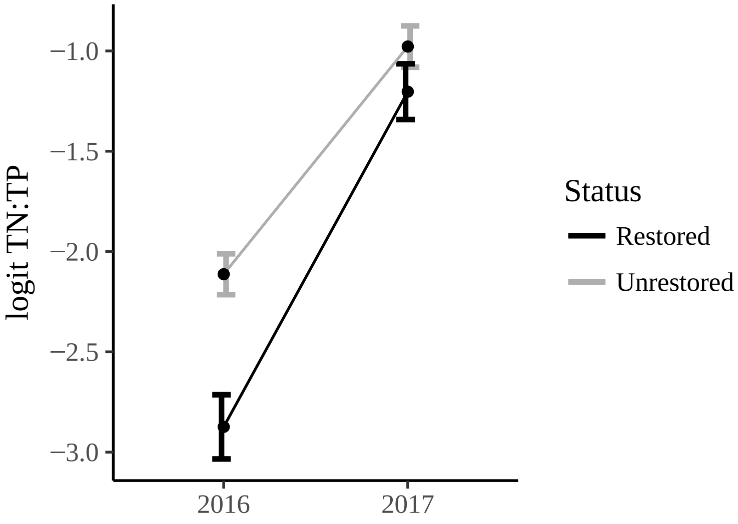

Figure 1. 13 Logit transformed TN:TP in restored and unrestored wetlands in 2016 and 2017.

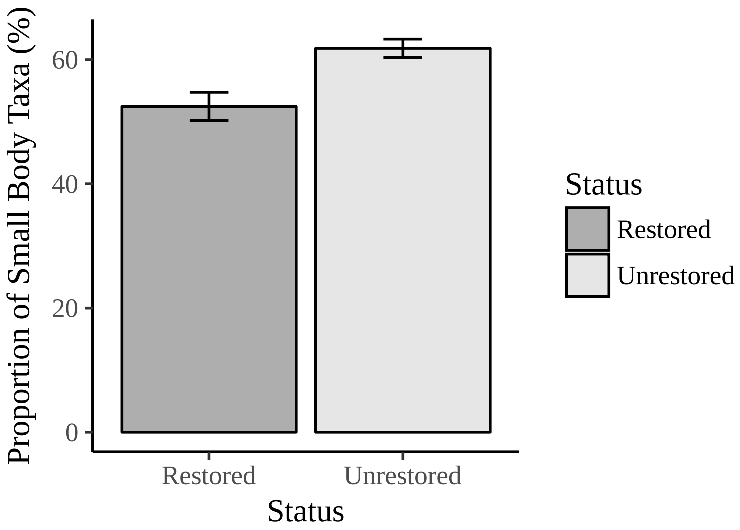

Figure 1. 14 Proportion of small body taxa in restored and unrestored wetlands in 2016 and 2017.

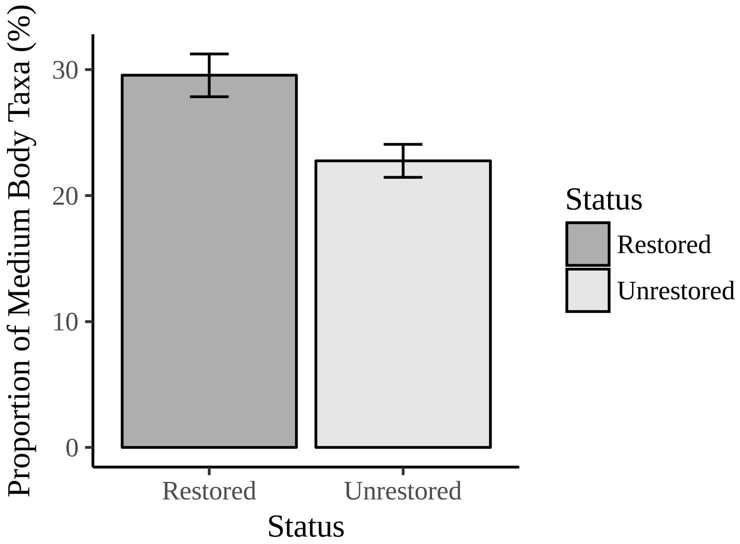

Figure 1. 15 Proportion of medium body taxa in restored and unrestored wetlands in 2016 and 2017.

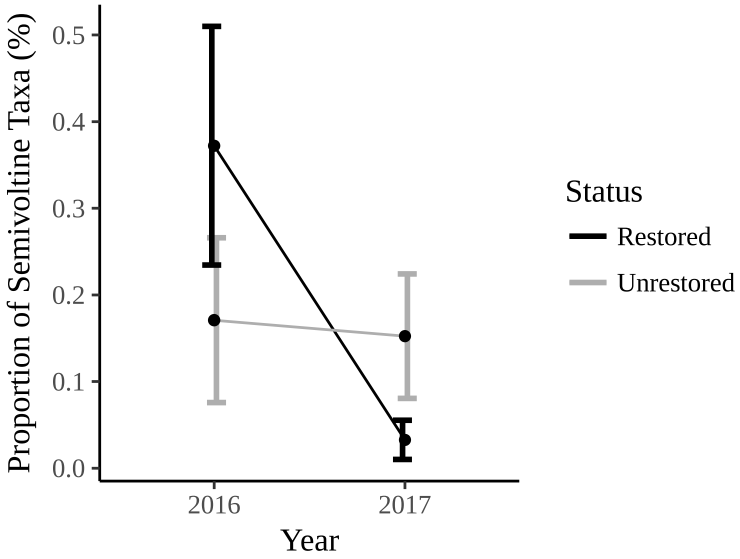

Figure 1. 16 Proportion of semivoltine macroinvertebrates in restored and unrestored wetlands in 2016 and 2017.

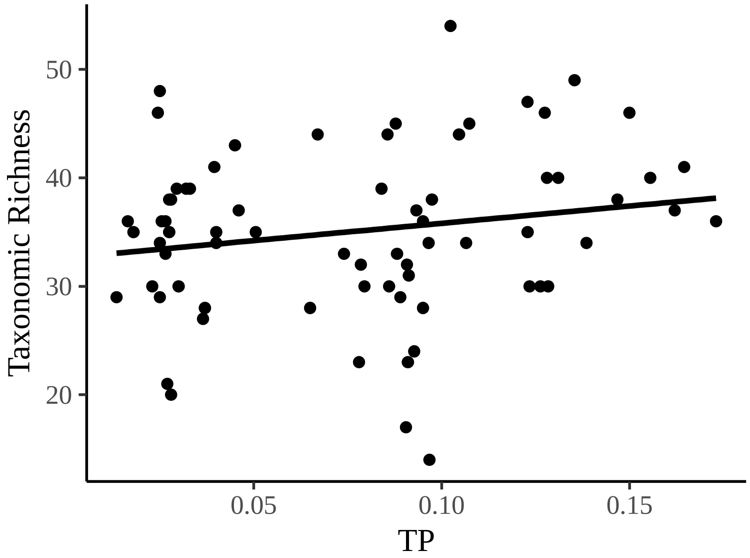

Figure 1. 17 Taxonomic richness and TP.

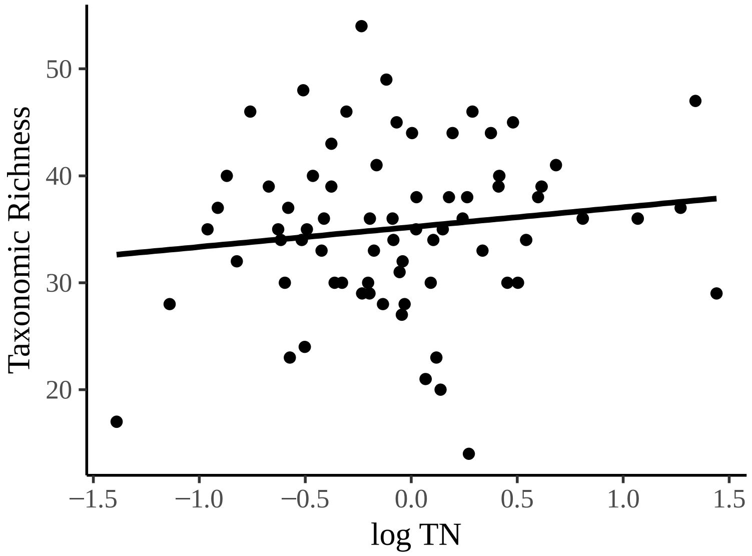

Figure 1. 18 Taxonomic richness and log transformed TN.

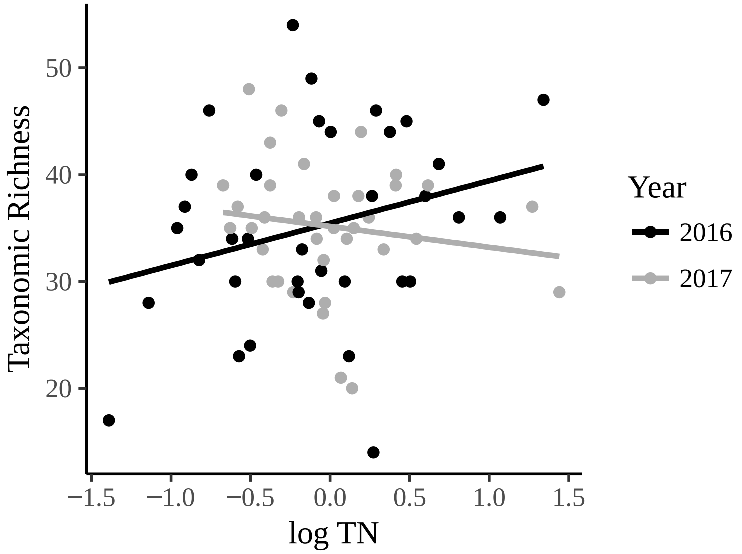

Figure 1. 19 Taxonomic richness and log transformed TN in 2016 and 2017.

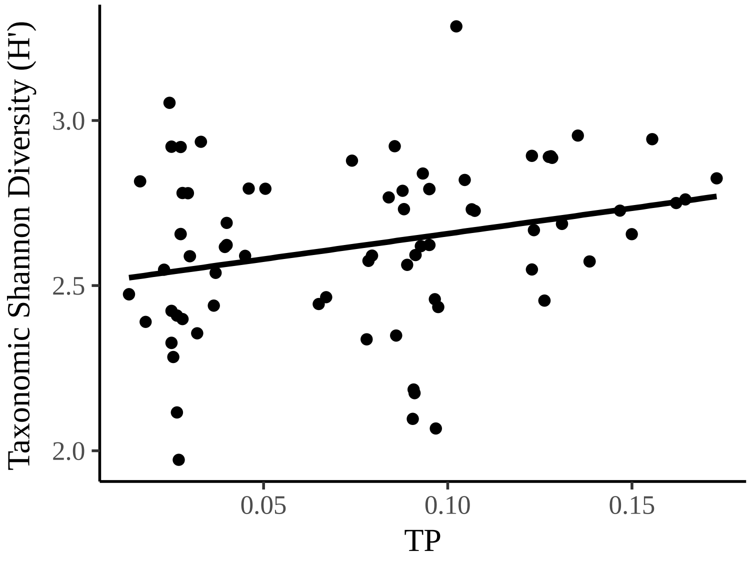

Figure 1. 20 Shannon Diversity (H') and TP.

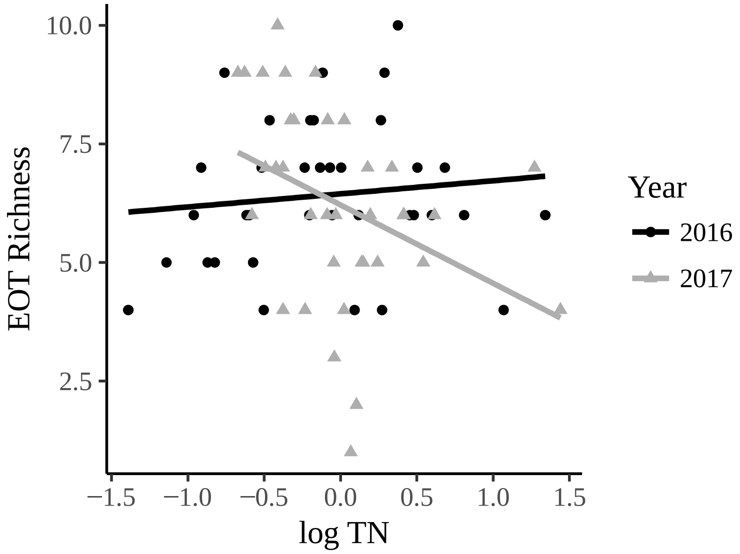

Figure 1. 21**.** EOT richness and log transformed TN in 2016 and 2017.

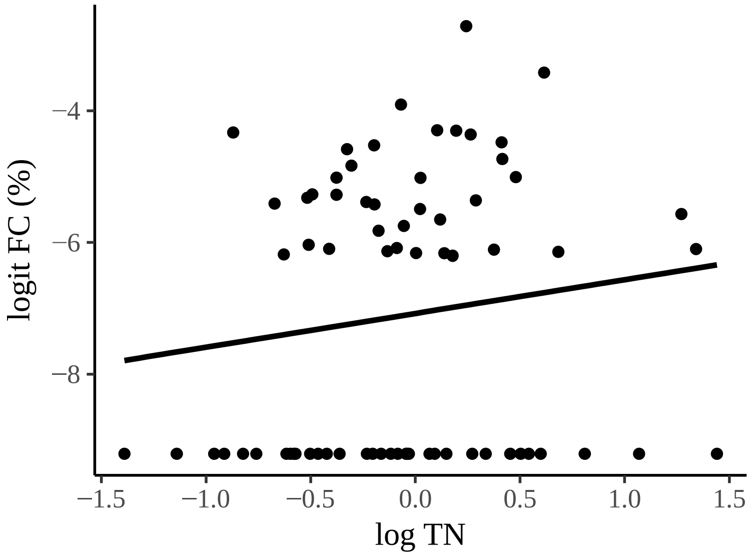

Figure 1. 22 Logit transformed proportion of FC and log transformed TN.

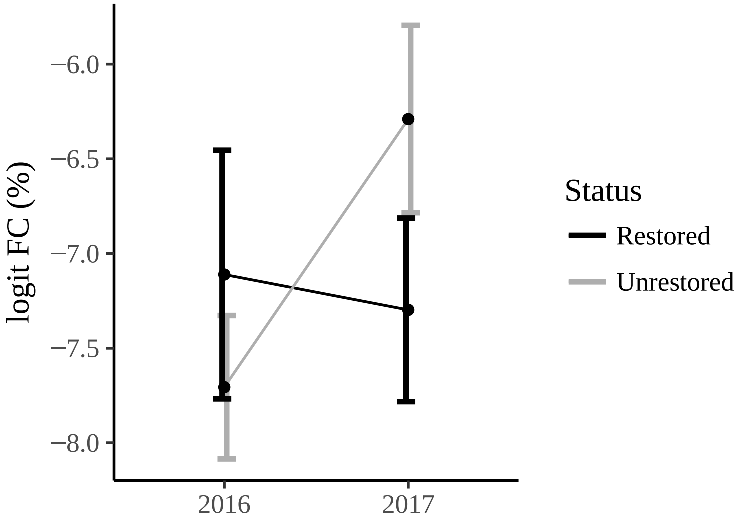

Figure 1. 23 Logit transformed proportion of FC in restored and unrestored wetlands in 2016 and 2017.

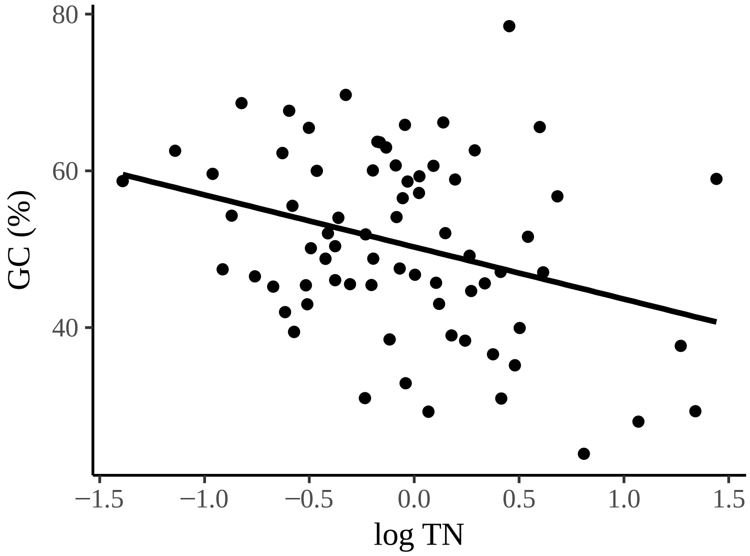

Figure 1. 24 Proportion of GC and log transformed TN.

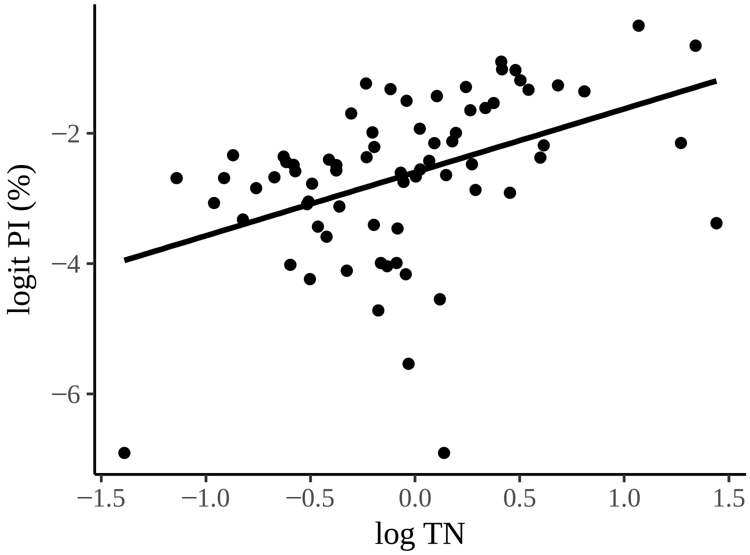

Figure 1. 25 Logit transformed proportion of PI and log transformed proportion of TN.

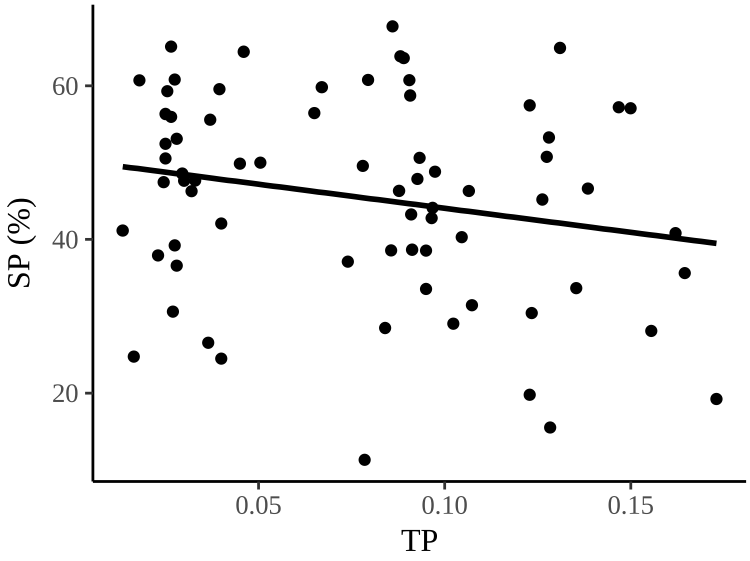

Figure 1. 26 Proportion of SP and TP.

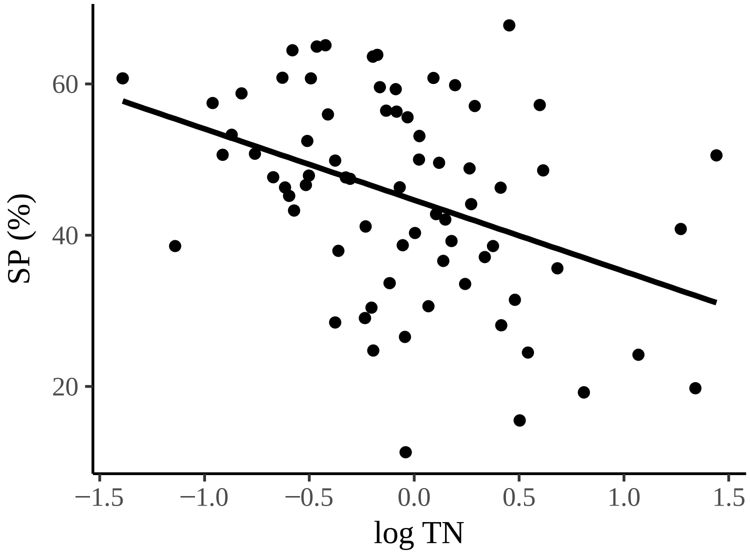

Figure 1. 27 Proportion of SP and log transformed TN.

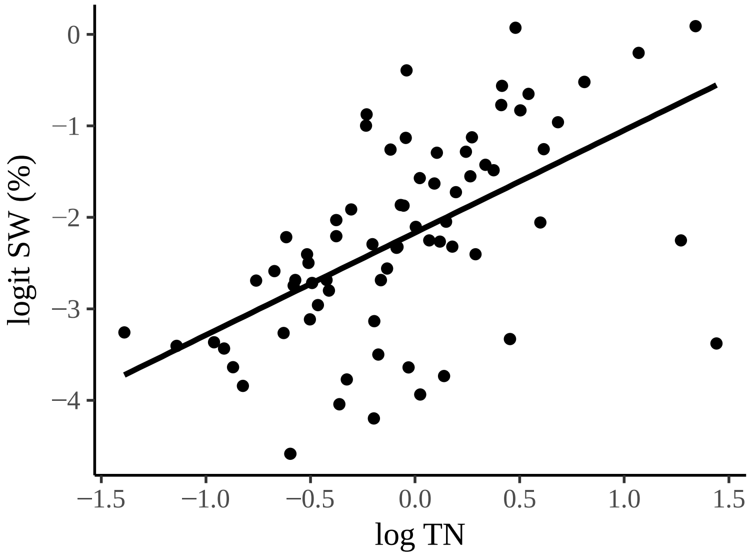

Figure 1. 28 Logit transformed proportion of SW and log transformed TN.

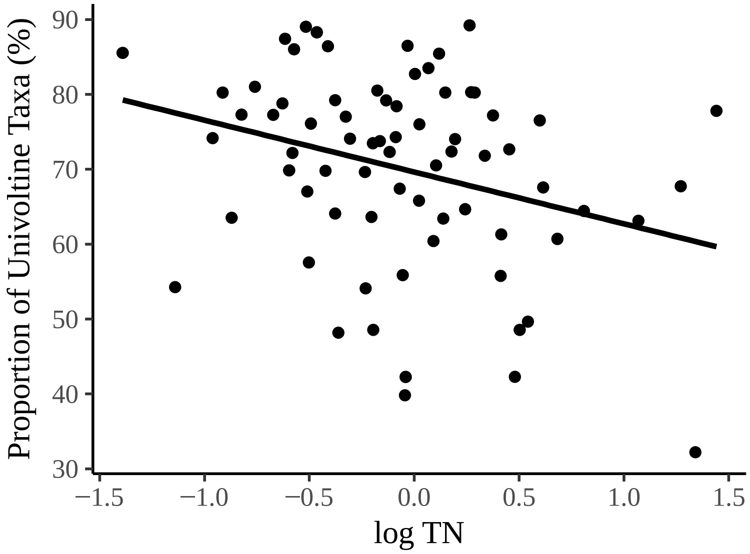

Figure 1. 29 Proportion of univoltine taxa and log transformed TN.

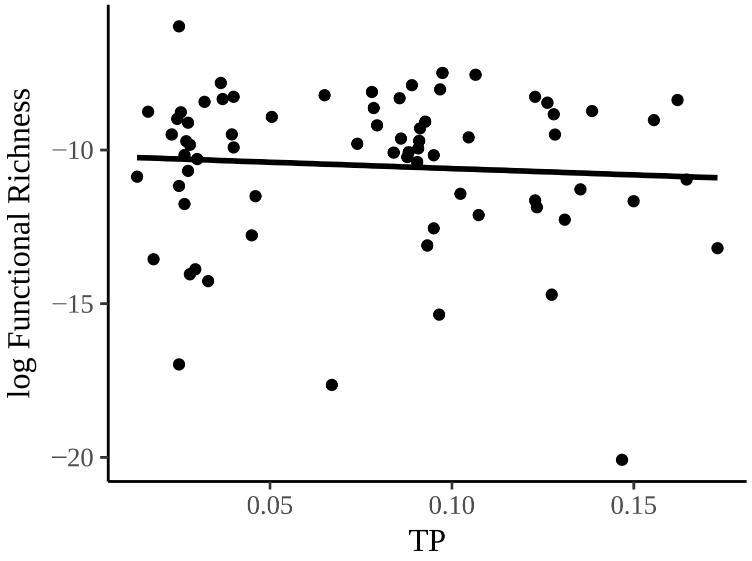

Figure 1. 30 Log transformed functional richness and TP.

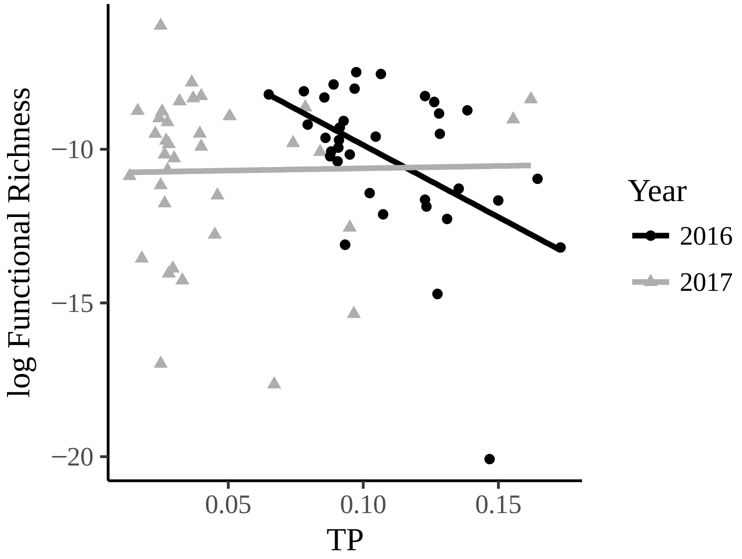

Figure 1. 31 Log transformed functional richness and TP in 2016 and 2017.

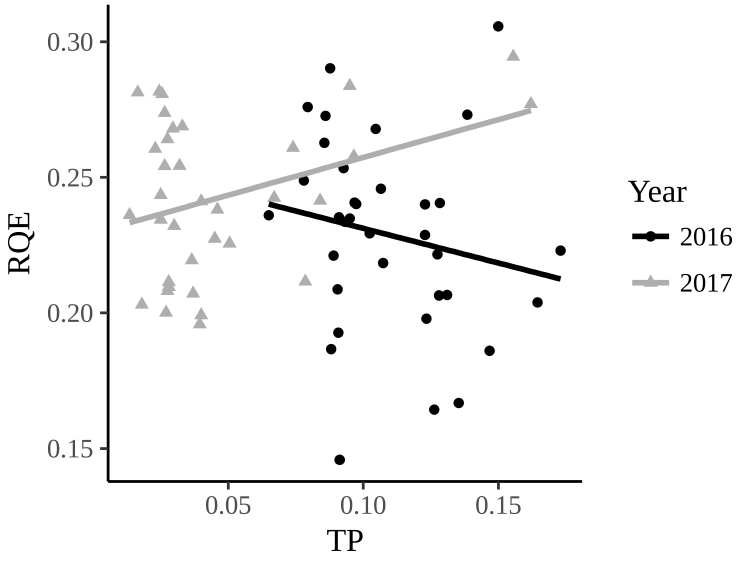

Figure 1. 32 RQE and TP in 2016 and 2017.

Chapter 2. The effect of hydrological restoration on nutrient concentrations and amphibian abundance and biomass in Lake Erie coastal wetlands

1. Introduction

Coastal wetlands along the western Lake Erie basin historically provided key ecosystem functions including water quality improvement and biodiversity support (Maynard and Wilcox 1997; Zedler and Kercher 2005). Most remaining coastal wetlands have been diked, severing hydrologic connection to Lake Erie and its tributaries and acting as barriers to the exchange of water, nutrients, and biota. Large-scale coastal wetland restoration is expected to improve water quality and reduce harmful algal blooms in Lake Erie (Mitsch et al 1989; Wang and Mitsch 1998; Mitsch and Wang 2000). In particular, stakeholders have collaborated to hydrologically reconnect approximately 2,397 acres of protected, diked wetlands in Ottawa National Wildlife Refuge (ONWR). Restoring wetland connectivity also has potential to reduce amphibian habitat fragmentation, one of the leading causes of amphibian decline in the Great Lakes region (Hecnar 2004). However, if hydrologic reconnection to an impaired watershed degrades wetland conditions, amphibians may be exposed to new stressors such as increased nutrient concentrations. Here, I examine whether coastal wetland restoration has an effect on amphibian abundance and biomass in hydrologically reconnected wetlands in ONWR.

Amphibians are the focus of increasing concern because they are experiencing global decline commonly linked to habitat loss and fragmentation, which is the leading cause of decline in the Great Lakes region (Semlitsch 2000; Hecnar 2004; Hamer and McDonnell 2008). While the status of amphibians in the Great Lakes prior to European settlement remains largely unknown, it is reasonable to assume that the historically large extensive wetlands supported species rich amphibian communities with large populations (Hecnar 2004). Post-European settlement, twenty-eight species of amphibians, including fifteen anurans, have be recorded in Lake Erie wetlands (Maynard and Wilcox 1997; Hecnar 2004). Of the fifteen anurans in the Lake Erie region, eleven have been recorded in the western basin (Herdendorf 1987; King et al 1997; Bird Studies Canada 2018) (Appendix C, Table C.1). Drainage for agricultural and urban development has left fragmented tracts of this once productive wetland ecosystem. Now, only 5% of natural Lake Erie coastal wetlands remain (Herdendorf 1987). Where amphibian dispersal does occur, there are often barriers imposed between fragments, such as roads, urban development, and agricultural land. Remaining coastal wetlands are largely managed by dikes, resulting in greater habitat isolation. Barriers to dispersal or migration between habitats can increase mortality and decrease connectivity among amphibian populations (Ashley and Robinson 1996; Hecnar 2004; Cosentino et al 2014). For example, Houlahan and Findlay (2003) examined amphibian species richness, abundance, and community composition in Ontario wetlands. They found that species richness and abundance were negatively correlated with road density and suggest that effective wetland conservation will require maintaining a heterogenous regional landscape (Houlahan and Findlay 2003). Removing dikes may have a positive impact on amphibian abundance and biomass if hydrologic reconnection leads to more heterogenous habitat. However, if hydrologic reconnection leads to an increase in nutrient concentrations (due to the agricultural landscape surrounding ONWR), amphibians may be exposed to new stressors in restored wetlands.

Restoring hydrological connections of coastal wetlands with the surrounding watershed is likely to increase nutrient inputs to the wetlands and alter nutrient concentrations. For example, removal of dikes and water control structures in the wetlands of the Everglades changed this system from its oligotrophic status to being enriched by phosphorus (Surratt et al 2012; Sullivan et al 2014). Similarly, restoration of hydrologic connectivity in the Chesapeake Bay, an impaired watershed characterized by elevated loads of phosphorus, nitrogen, and sediment, led to increased nutrient inputs in reconnected wetlands (Jordan et al 2000; Wolf et al 2013). Early studies at ONWR show effects of hydrologic reconnection on nutrient concentrations. Specifically, Kowalski et al. (2014b) compared water quality in diked and connected wetlands in ONWR. ONWR is located at the mouth of the Maumee River, which is currently listed as an Area of Concern (AOC) in part due to nutrient impairment (Ohio EPA 2012). Compared to diked wetlands, connected wetlands had higher nitrate concentrations that indicated impaired water quality (Kowalski et al 2014b). Following reconnection of one diked wetland in ONWR, Kowalski et al (2014a) then compared water quality pre- and post-hydrologic reconnection. Post-reconnection, water chemistry began to reflect impaired conditions in Crane Creek and Lake Erie (Kowalski et al 2014a). If hydrologic reconnection results in higher nutrient concentrations, there may be adverse effects on amphibian abundance and biomass in restored ONWR wetlands.

Amphibians have several physiological and ecological traits that put them at risk to pollutants. Their thin, semi-permeable skin readily absorbs moisture and facilitates the uptake of pollutants when they are present in aquatic habitats (Price et al 2007). Amphibians rely on aquatic habitats for at least some part of their life cycle. Larvae are more at risk than adults because they are confined to the aquatic environment and species with relatively long larval periods, including American bullfrogs (*Rana catesbeianus)* and green frogs (*Rana clamitans*), have comparatively prolonged exposure (Semlitsch 2000; Price et al 2007). Similarly, adults that hibernate in aquatic environments, such as American bullfrogs, green frogs, and Northern leopard frogs (*Rana pipiens*), are at risk of prolonged exposure to pollutants. Specifically, pollutants such as nitrogen-based fertilizers may be contributing to amphibian population decline. Several studies have shown adverse sublethal and lethal effects of nitrogen concentrations in laboratory experiments and in field studies on amphibians (Baker and Waights 1994; Hecnar 1995; Bishop et al 1999; Rouse et al 1999; Houlahan and Findlay 2003; Knutson et al 2004). Nitrogen occurs in aquatic environments as ammonium ion, ammonia, nitrite and nitrate. Nitrate is the least toxic of these forms, but it occurs at the highest concentrations and is the most stable form of nitrogen in the aquatic environment (Rouse et al 1999). Therefore, studies that examine the effects of nitrogen on amphibians usually focus on nitrate. Nitrate concentrations can reach high levels in wetlands within agricultural landscapes in particular that result in mortality and reduced feeding, growth rate, and swimming ability in larvae (Baker and Waights 1994; Hecnar 1995). Small, lethargic larvae are at greater risk of predation. Additionally, reduced grown rate during the larval stage can result in failure to exit a deteriorating aquatic environment, prolong time taken to reach maturity, and reduce body size at maturity. These direct effects of nitrate are associated with low reproductive potential and have potential consequences at the population level (Baker and Waights 1994; Hecnar 1995). Nitrate has been detected in the Great Lakes at levels reported to exhibit lethal and sublethal effects (Rouse et al 1999; Ohio EPA 2012). Nitrate levels are likely higher in AOCs that experience nutrient impairment, including the Maumee River AOC. Tozer (2013) reported that particular amphibians had higher occupancy in wetlands located outside of Great Lakes AOC boundaries compared to wetlands within AOCs. Lower occupancy may be related to elevated nitrate levels. Indeed, nitrate concentrations in Crane Creek exceed the target of 1.0 mg/L set for warm water habitat by the Ohio EPA. Samples collected in in Crane Creek in 2011 had nitrate concentrations above 6.0 mg/L (Ohio EPA 2012). Lethal and sublethal effects in amphibians are detected at nitrate concentrations between 2.5 and 385 mg/L (Rouse et al 1999). However, no studies have examined whether changes in nutrient concentrations impact amphibians in ONWR. If hydrologic reconnection increases nitrate concentrations, amphibian abundance and biomass may decline in restored wetlands.

Elevated nutrient concentrations of nitrogen and phosphorus can also have either positive or negative indirect effects on amphibians by altering habitat through changes in wetland vegetation. Typically, wetland plants provide refuge for larvae and adults as well as breeding and oviposition sites (Egan and Paton 2004; Hamer and McDonnell 2008). Added nutrients can stimulate growth of emergent vegetation. Greater vegetation cover may lead to greater amphibian abundance and biomass, particularly if added habitat provides refuge and breeding and oviposition sites. For example, Egan and Paton (2004) observed greater annual breeding effort and more egg masses in wetlands with more complex habitat structure. Studies have also found that species richness and abundance were positively correlated with the amount of emergent vegetation (Burne and Griffin 2005; Pearl et al 2005). However, an increase in emergent vegetation can reduce open water and submerged vegetation zones and subsequently reduce habitat heterogeneity that have adverse effects for amphibians (Maynard and Wilcox 1997). Under conditions of nutrient enrichment, weedy or non-native invasive species such as common reed (*Phragmites australis*) and narrow-leaved cattail (*Typha angustifolia*) may outcompete less aggressive native taxa (Chambers et al 1999; Blann et al 2009). Common reed biomass was shown to negatively impact tadpole development by extending development period (Perez et al 2013). Another study found that the spread of common reed contributed to ongoing loss of adult breeding habitat and population declines for Fowler's toads (Greenberg and Green 2013). Amphibian abundance and biomass may be affected by changes in wetland vegetation if restoring hydrologic connection increases nutrient concentrations in reconnected ONWR wetlands.

Nutrients also have indirect effects on amphibians by altering food quality and quantity. Amphibian larval diets vary widely across taxa and environments, but are largely dependent on vascular plants, algae, dissolved organic matter, and detritus. Added nutrients can influence growth rates and size at metamorphosis by increasing food quantity and quality (Dodds et al 2002; Stephens et al 2013; Barrett et al 2017). For example, leaf litter quality (i.e. carbon, nitrogen and phosphorus content) were shown to influence the abundance of primary producers and led to increased amphibian mass at metamorphosis in wetlands (Stephens et al 2013). However, amphibian larvae may only benefit from nutrient inputs up to the level of enrichment. Nutrient enrichment can stimulate tall emergent plant growth, shade algal growth, and subsequently limit a primary larval food resource. Perez et al. (2013) observed this subsidy-stress response in wetlands. Under high common reed densities, phytoplankton groups important to amphibian larval diet were less abundant (Perez et al 2013). Additionally, detrital material from decaying reeds and other invasive plants can produce toxins that limit larval growth and survival (Bucciarelli et al 2014). Therefore, restoring hydrologic connection may indirectly affect amphibians in ONWR wetlands if added nutrients influence food quality or quantity.

Beginning in 2011, 5 diked wetlands in ONWR were hydrologically reconnected to Lake Erie and Crane Creek. ONWR offers a unique opportunity to evaluate restoration outcomes of reconnecting diked wetlands to an impaired watershed. In addition to the reconnected wetlands, many of the wetlands remained diked and provide the opportunity to compare amphibian abundance and biomass between diked and reconnected wetlands. Thus, I examined the association of hydrologic reconnection on nutrient concentrations, algal resources (measured as ash-free dry mass) and habitat resources, and amphibian abundance and biomass. Specifically, my objectives were to 1) quantify and compare nutrient concentrations, algal and habitat resources, and amphibian abundance and biomass in restored and unrestored wetlands, and 2) examine relationships between nutrient concentrations, algal and habitat resources, and amphibian abundance and biomass. I predicted that I would observe any species that have been recorded within or near ONWR by the Great Lakes Marsh Monitoring Program (Appendix C, Table C.1). If hydrologic reconnection increased nitrate concentrations, I predicted that amphibian abundance and biomass would decline in restored wetlands. If hydrologic reconnection increased total phosphorus and nitrogen concentrations, I predicted that amphibians would be indirectly affected by changes to algal and habitat resources in restored wetlands.

2. Methods

2.1 Study Site

The ONWR wetland complex is a 2,397-acre freshwater estuary complex fed by Crane Creek, a small Maumee River tributary. Crane Creek flows into the wetland complex from the west and exits to Lake Erie through a permanent channel between a break in the shoreline dikes on the eastern boundary where Crane Creek and Lake Erie meet to form a freshwater estuary. Wetlands are characterized by tall, emergent vegetation stands (e.g., narrowleaf cattail *Typha angustifolia*) that populate the edges and form stands in the centers of many wetland units, while floating-leaf assemblages (e.g., American lotus *Nelumbo lutea*) extend further from shore. In many wetlands, dense submerged aquatic vegetation mats also extend further form shore. The United States Fish and Wildlife Service manages ONWR via dikes and water control structures and pumps with the primary goals to maximize migratory bird and waterfowl habitat and minimize colonization of invasive plants. Beginning in 2011, five diked units have been hydrologically reconnected to Crane Creek and Lake Erie. I studied the five restored wetlands and eight diked, unrestored wetlands (Figure 2.1).

Unrestored wetlands are hydrologically isolated from Crane Creek and Lake Erie by earthen and rock dikes. Water levels in diked units are artificially managed with occasional drawdowns by pumping creek water in and out depending on management goals. Precipitation and evapotranspiration also contribute to water level changes in diked units. Unrestored wetlands include MS3, Pool 3, MS4, MS5, MS7A, MS8N, Pool 9E. All unrestored wetlands are adjacent to Crane Creek and Lake Erie and commonly take in water during high water events. In 2011, MS5 water levels were drawn down to maintain migratory bird and waterfowl habitat. MS8B has a possible connection to Crane Creek through a water control structure on the northwestern edge connecting to MS8A, but the structure was not active at the time of this study. Water was exchanged periodically by pumping between MS8B and other wetlands.

Restored wetlands are connected directly or indirectly to Crane Creek and Lake Erie by water control structures. ONWR manages water level by occasionally pumping between restored units and Crane Creek, but water levels in restored units are mainly driven by fluctuations in Crane Creek and Lake Erie. Annual water levels in Lake Erie can fluctuate greatly (>1m) depending on antecedent climate. Seasonal water levels can also fluctuate greatly during seichal events. Water control structures allow for water, nutrients, sediment, and biota to exchange between restored wetlands, Crane Creek, and Lake Erie with the exception of some large fishes due to the presence of carp grates. Carp grates are removed after carp spawning season, allowing other large fish to access restored wetlands periodically. Restored wetlands include Pool 2A, Pool 2B, Pool 2C, MS8A, and Pool 1. Pool 2B is adjacent to Crane Creek and has had a continuous open connection since 2011 through a water control structure on the northern edge. Pool 2C is indirectly connected to Crane Creek through a water control structure on the eastern edge of Pool 2C and the east ditch maintains a continuous connection to Crane Creek. Pool 2C had a continuous connection to Crane Creek between 2011-2013. In 2014 and 2015, it was drawn down for construction. In 2017, it was drawn down again for invasive plant management. Pool 2A is adjacent to Crane Creek and has maintained an open continuous connection to Crane Creek indirectly through a water control structure between Pool 2A and Pool 2B. MS8B is adjacent to Crane Creek and has a direct but limited connection through a permanent pump structure on the northwestern edge. The structure was opened periodically (days-weeks at most) for water exchange in 2016 and 2017. Pool 1 is directly connected to Crane Creek by a water control structure on the northwestern edge and has maintained an open continuous connection since May 2017.

2.2 Data Collection

To compare nutrient concentrations and algal biomass (measured as ash-free dry mass) between restored and unrestored wetlands, I took bi-weekly in situ water samples for eight weeks (May—August). I collected one sample per wetland in open water (n=40/restored wetlands, n=56/unrestored wetlands). Although water samples were collected in 2016 and 2017, I only used 2017 samples because they were collected during the same time period as amphibian data. Water samples were collected with acid-washed 250 mL polyethylene bottles, filtered through a 0.45 μm membrane and frozen. Within 3 weeks of collection, samples were transported the Ohio State University Service Testing and Research Laboratory for Flow Injection Analysis for nitrate-N and total phosphorus. AFDM (mg/cm^2) was measured by filtering a separate water sample through a 0.45 µm membrane filter and drying the filtered material for 24 hours or until the biomass reached a constant mass. AFDM was measured within a week of collection. I also measured temperature (ºC), pH, conductivity (µS/cm), and DO (%) measured on a multi-meter probe (YSI Model 665) turbidity (NTUs) measured on a handheld spectrophotometer (Hach Turbidimeter), and depth (cm) in three vegetation zones (emergent, submerged, and floating) (n=120/restored wetlands, n=168/unrestored wetlands).

To compare amphibian habitat in restored and unrestored wetlands, I quantified wetland plant richness and biomass. Plant richness was measured in June to facilitate better species identification with plant reproductive parts. For a more accurate representation of overall plant species richness of restored and unrestored wetlands, a stratified sampling approach was taken in which each of the three major wetland vegetation zones (emergent, submerged, and floating) were sampled in all 12 wetlands (Lougheed et al 2001). I visually assessed each wetland from the edge and chose three sampling locations with distinct emergent, submerged and floating vegetation that were representative of the wetland. Within each zone, three 0.5 m^2 quadrats were used to collect plant richness in restored (n=45) and unrestored (n=63) wetlands. Quadrats were placed at least one meter from the vegetation zone boundaries, and were placed two meters apart from other quadrats to avoid trampling vegetation (Uzarski et al 2015). Vouchers were collected for species that were unidentifiable in the field, then were pressed and dried using a forced-air space heater (Blanco et al 2006). Dichotomous keys were used to identify specimens in the laboratory to genus or species (Voss and Reznicek 2012; Smith 2017). Above-ground plant biomass (g/m^2) of emergent vegetation was sampled in August when peak biomass typically occurs (Julie and Fennessy 2001). Plant biomass was harvested from three 0.5 m^2 quadrats in restored (n=15) and unrestored (n=21) wetlands. Harvested plant material was oven-dried in paper bags for one week at 65°C until plant biomass reached a constant mass.

To compare amphibian abundance and biomass between restored and unrestored wetlands, I set 6 minnow traps lined with 1 mm mesh with 1.5” openings and 3 large collapsible Promar 6 mm mesh traps with 5” openings at 14 locations in restored (n=45) and unrestored (n=63) wetlands monthly from May-August 2017 (Figure 2.1). Traps were placed evenly along one or more edges of each wetland adjacent to one of three major vegetation zones (emergent, submerged, and floating) so that each vegetation zone was associated with 3 traps (1 minnow trap and 2 large mesh traps) for a total of 9 traps in each wetland. All traps were set for 19-24 hours. Species, sex, life stage, weight (g), and size (cm) were recorded for each individual. For tadpoles and metamorphs, snout-vent-length and total length were recorded. For juveniles and adults, total length was recorded. Catch per unit effort (CPUE) was used to compare amphibian abundance in traps.

To further compare amphibian abundance between restored and unrestored wetlands, I conducted monthly call surveys at the 14 locations where I set traps from May-August 2017 following the standard operating procedure from the Coastal Wetland

Sampling Program led by the Institute for Great Lakes Research. Call surveys were conducted on evenings with little or no wind, began ½ hour after sunset, and ended 4 ½ hours after sunset. In May, call surveys were conducted once at each location in restored (n=6) and unrestored (n=8) wetlands. In June and July, call surveys were conducted 3 times per location in restored (n=18) and unrestored (n=24) wetlands. When possible, points were separated by at least 500 m. Before conducting each call survey, we waited quietly for 2 minutes. Each call survey was conducted for 3 minutes. Percent cloud cover, Beaufort wind scale (Appendix, Table C.2), noise code (Appendix, Table C.3), weather (Appendix, Table C.4) and air temperature (°C) were recorded before each call survey. Species calling codes (Appendix, Table C.5) were recorded within the distance intervals <50, 50-100m, >100m. Number of individuals were estimated when possible. I compared call codes of each species detected during call surveys in restored and unrestored wetlands.

2.3 Statistical Analysis

To compare nutrient concentrations between restored and unrestored wetlands, I used linear mixed effects models:

$$Nutrient \sim Status + Time + Status*Time + (1|Site)$$

Where nutrient represents either nitrate-N or total phosphorus, status represents restored or unrestored wetlands. Nutrient is the dependent variable, status, time and the interaction term status*time are fixed effects, and site is a random effect.

To understand whether nutrients effect amphibian algal resources, I used linear mixed effects models to test whether algal biomass measured as AFDM varied with nutrient concentrations:

AFDM ~ Status + Nitrate-N (OR) TP + (1|Site)

AFDM was calculated as:

$$AFDM = \frac{\left(W_a - W_f\right) - W_{ash}}{A_{t/r}}$$

Where W_a = dried algae on filter (mg), W_f = filter weight (mg), and $A_{t/r}$ = area of filter (cm^2), and W_{ash} = material on filter (mg) after ashing. Additionally, to understand whether nutrients effect amphibian wetland plant resources, I used linear models to test whether plant richness or plant biomass varied with nutrient concentrations:

Plant Richness (OR) Plant Biomass ~ Status + Nitrate-N (OR) TP

Nutrient data corresponding to the last sampling trip was used.

To compare amphibian abundance measured as CPUE between restored and unrestored wetlands, I used linear mixed effects models:

CPUE ~ Status + (1|Site)

Where CPUE was calculated as:

$$CPUE = \left(\frac{Individuals}{Hours}\right)(Traps)$$

I compared CPUE of tadpoles from each species collected and total tadpole CPUE in restored and unrestored wetlands. I did not use CPUE to compare adult abundance because captures were low. CPUE did not change over the three sampling periods, therefore time was not included in any of the models. American toad (*Bufo americanus*) was not considered in CPUE calculations because they were found in one wetland during one sampling trip.

To compare amphibian abundance measured through the call surveys between restored and unrestored wetlands, I used linear mixed effects models:

*Call Code ~ Status + Time + Status*Time + (1|Site/Survey)*

Where call code represents adult calling of individual species. Call survey was nested within site as a random effect to control for the repeated survey measures at each site. Call code was calculated as the sum of each species' calling codes at each site. Call code reflects a range from 0-9, where 0 = no individuals calling and 9 = species heard calling at all 3 distances at a calling code of 3.

To compare amphibian biomass between restored and unrestored wetlands, I used linear mixed effects models:

Amphibian biomass ~ Status + (1|Site)

Where amphibian biomass represents the biomass of individual species collected. Amphibian biomass did not change over the three sampling periods, therefore time was not included in any of the models. Biomass was calculated as the sum of the individual wet weight at each site. I did not use biomass to compare adults because captures were low. American toad was not considered in biomass calculations because they were found in one wetland during one sampling trip.

To examine whether nitrate-N or amphibian resources explained variation in tadpole abundance and biomass within restored and unrestored wetlands, I used linear mixed effects models:

$$\mathit{Amphibian\ Metric} \sim \mathit{Status} + \mathit{nitrate\text{-}N}\ (\mathit{OR})\ \mathit{AFDM} + (1|\mathit{Site})$$

Where amphibian metric represents tadpole CPUE or biomass measures. Nitrate-N and AFDM measurements corresponding to the two weeks closest to when amphibians were collected were averaged. Additionally, I used linear models to examine whether plant richness or plant biomass explained variation in tadpole abundance and biomass within restored and unrestored wetlands:

$$\mathit{Amphibian\ Metric} \sim \mathit{Status} + \mathit{Plant\ Richness}\ (\mathit{OR})\ \mathit{Plant\ Biomass}$$

Where amphibian metric represents tadpole CPUE or biomass measures. Tadpole CPUE and biomass were averaged across sampling trips to create a variable representing overall site CPUE and biomass for each species.

To examine whether amphibian resources explained variation in adult abundance within restored and unrestored wetlands, I used linear models:

$$\mathit{Amphibian\ Metric} \sim \mathit{Status} + \mathit{Plant\ Richness}\ (\mathit{OR})\ \mathit{Plant\ Biomass}$$

Where amphibian metric represents adult calling codes. Calling codes were averaged across sampling trips to create a variable representing overall site calling codes for each species.

All statistical analyses were conducted in R version 3.4.2 (R Development Core Team 2017). The lme4 package was used to run linear mixed effects models (Bates et al 2014). P-values were obtained using the lmerTest package (Kuznetsova et al 2017). The marginal (R^2m) and conditional (R^2c) R^2 values were obtained using the r.squaredGLMM function. All dependent variables were tested for normality and were transformed if they did not meet normality assumptions. All dependent variables were tested for normality and were transformed if they did not meet normality assumptions. Continuous variables including nitrate-N, TP, and tadpole CPUE and plant biomass were log-transformed. Additionally, the continuous variable AFDM was square root transformed. The standard errors of the means were calculated and used to estimate variability of each parameter.

3. Results

3.1 Description of amphibian assemblage

I collected and identified a total of 62 individuals in restored wetlands and 238 individuals in unrestored wetlands from traps. Four species including American bullfrog, Northern leopard frog, green frog, and American toad were collected in traps (Appendix, Table C.7), and an additional species, gray treefrog (*Hyla versicolor)* was heard during call surveys (Appendix, Table C.9). American bullfrog was the most abundant species trapped in restored wetlands (n=41) and American toad was the most abundant species trapped in unrestored wetlands (n=117). Tadpoles were more abundant in traps than adults in restored and unrestored wetlands. American bullfrog and American toad were the most frequent calling amphibians in restored wetlands. American bullfrog and Green frog were the most frequent calling amphibians in unrestored wetlands. Northern leopard frogs were not detected during call surveys.

3.2 Effect of restoration on nutrients

Nitrate-N was higher in restored wetlands than unrestored wetlands ($R^2m=0.33$, $R^2c=0.46$, p=0.004) (Table 2.1) (Figure 2.2) (Appendix, Table C.6). In both restored and unrestored wetlands, nitrate-N decreased over time (p=1.46E-09). There was a significant interaction between status and time (p=0.002). The interaction indicated that the effect of status on nitrate-N depended on time. Specifically, nitrate-N was higher in restored wetlands during the first three sampling events, but similar in restored and unrestored wetlands during the remaining five sampling events.

TP was similar in restored and unrestored wetlands ($R^2m=0.004$, $R^2c=0.27$, p=0.78) and did not vary significantly over time (p=0.50) (Table 2.1) (Appendix, Table C.6). The interaction between status and time was not significant (p=0.61). Mean TP was 0.04 mg/L (±0.01) and 0.05 mg/L (±0.004) in restored and unrestored wetlands respectively.

3.2 Effect of nutrients on amphibian resources in restored and unrestored wetlands

AFDM was lower in restored wetlands than unrestored wetlands when nitrate-N ($R^2m=0.12$, $R^2c=0.27$, p=0.04) or TP ($R^2m=0.15$, $R^2c=0.31$, p=0.047) was included in the model and did not vary significantly with nitrate-N or TP (Table 2.2) (Figure 2.3). Plant richness and plant biomass were similar in restored and unrestored wetlands and did not vary significantly with nitrate-N or TP (Table 2.3A-B).

3.4 Effect of restoration on amphibian abundance and biomass

Total tadpole abundance measured as CPUE was similar in restored and unrestored wetlands (Table 2.4) (Appendix C, Table C.8). Additionally, all species-specific tadpole abundance measured as CPUE including American bullfrog, Northern leopard frog, and green frog were similar in restored and unrestored wetlands (Table 2.4) (Appendix C, Table C.8).

American bullfrog call codes were similar in restored and unrestored wetlands **(**$R^2m=0.43$, $R^2c=0.60$, p=0.06) (Table 2.5) (Appendix, Table C.9), but call codes decreased over time ($p=5.68E-06$) (Figure 2.4). The interaction between status and time was not significant ($p=0.16$).

Green frog call codes were lower in restored wetlands than unrestored wetlands **(**$R^2m=0.22$, $R^2c=0.34$, $p=0.01$) (Table 2.5) (Figure 2.5) (Appendix, Table C.9). Green frog call codes did not vary over time ($p=0.64$). The effect of time depended on wetland status ($p=0.04$) (Figure 2.6). Calls peaked in unrestored wetlands and were higher in unrestored wetlands than restored wetlands mid-study.

American toad call codes were similar in restored and unrestored wetlands **(**$R^2m=0.20$, $R^2c=0.32$, $p=0.06$) (Table 2.5) (Appendix, Table C.9). American toad call codes increased over time ($p=2.70E-05$) (Figure 2.7). The effect of time depended on wetland status ($p=0.01$). Calls increased over time in restored and unrestored wetlands. Additionally, restored wetlands had more calls than unrestored wetlands over time.

Gray treefrog call codes were similar in restored and unrestored wetlands **(**$R^2m=0.17$, $R^2c=0.43$, $p=0.39$) (Table 2.5) (Appendix, Table C.9). Gray treefrog call codes increased over time ($p=1.26E-04$) (Figure 2.8). The interaction between status and time was not significant.

Species-specific tadpole biomass was similar in restored and unrestored wetlands for all species collected in traps including American bullfrog, Northern leopard frog, and green frog (Tables 2.4) (Appendix, Table C.10).

3.5 Effect of nutrients, algal biomass, and wetland plant habitat on amphibian abundance and biomass in restored and unrestored wetlands

Total tadpole CPUE was similar in restored and unrestored wetlands and did not vary significantly with nitrate-N, AFDM (Table 2.7), or plant richness (Table 2.8). Total tadpole CPUE significantly decreased with increasing plant biomass (R^2=0.42, p=0.03) (Figure 2.9). American bullfrog tadpole CPUE was similar in restored and unrestored wetlands and did not vary significantly with nitrate-N (Table 2.6), AFDM (Table 2.7), or plant richness (Table 2.8). American bullfrog tadpole CPUE significantly decreased with increasing plant biomass (R^2=0.51, p=0.02) (Table 2.9) (Figure 2.10). Northern leopard frog and green frog tadpole CPUE were similar in restored and unrestored wetlands and did not vary significantly with nitrate-N (Table 2.6), AFDM (Table 2.7), plant richness (Table 2.8), or plant biomass (Table 2.9).

American bullfrog, green frog, American toad, and gray treefrog call codes were similar in restored and unrestored wetlands and did not vary significantly with plant richness (Table 2.8) or plant biomass (Table 2.9).

American bullfrog tadpole biomass was similar in restored and unrestored wetlands and did not vary significantly with nitrate-N (Table 2.6), AFDM (Table 2.7), or plant richness (Table 2.8). American bullfrog tadpole biomass significantly decreased with increasing plant biomass (R^2=0.53, p=0.02) (Table 2.9) (Figure 2.11). Northern leopard frog and green frog tadpole biomass were similar in restored and unrestored wetlands and did not vary significantly with nitrate-N (Table 2.6), AFDM (Table 2.7), plant richness (Table 2.8), or plant biomass (Table 2.9).

4. Discussion

4.1 Effect of restoration on nutrients

Overall, nitrate-N concentrations were higher in restored wetlands in comparison to unrestored as predicted but depended on time. Specifically, restored wetlands had higher nitrate-N concentrations than unrestored wetlands from May through the first week of June (Figure 2.2). From the last week of June through the end of August restored wetlands had similar concentrations to unrestored wetlands. These higher nitrate-N concentrations in late spring within the reconnected wetlands may suggest that restored wetlands experienced higher runoff following high precipitation events that occurred in early spring of 2017. When high precipitation events occur in early-spring (February), nutrient concentrations can spike in late-spring (May). Agricultural practices can exacerbate this effect if high precipitation events in early-spring correspond with autumn fertilizer application (Michalak et al 2013). Indeed, water year (wy) 17 had high precipitation events February through March. Additionally, the Ohio EPA reported that water year 17 had higher precipitation events and subsequently higher total nitrogen loading in the Maumee River watershed relative to previous years (Ohio EPA 2018). These conditions contributed to one of the largest HABs on record in 2017 (NOAA 2018). However, TP did not follow a similar trend. TP concentrations were similar in restored and unrestored wetlands throughout the sampling period and did not show seasonal variation. The Ohio EPA reported that water year 17 also had higher total phosphorus loading in the Maumee River watershed relative to previous years, but this was not reflected in restored wetlands. Therefore, it is difficult to interpret whether hydrologic reconnection had an impact on nutrient concentrations given that restored wetlands had higher nitrate-N concentrations but did not differ in TP.

4.2 Effect of nitrate-N on amphibian tadpole abundance and biomass

Although restoration led to higher nitrate-N concentrations, nitrate-N did not affect tadpole abundance or biomass of any species collected in restored or unrestored wetlands, likely because concentrations were too low to have lethal or sublethal effects. Rouse et al. (1999) reported that lethal effects of nitrate on amphibians ranged from 14 to 385 mg/L, while sublethal developmental effects ranged from 2.5 to 10 mg/L. On average, nitrate-N levels in ONWR were well below this threshold. Both restored and unrestored wetlands had an average nitrate-N concentration of 0.14 mg/L. While nitrate-N in Crane Creek has been reported at concentrations above 6.0 mg/L, the highest detected nitrate-N level in restored wetlands was 2.2 mg/L. Therefore, hydrologic reconnection did not appear to increase nitrate-N levels above concentrations that would directly affect amphibians.

4.3 Amphibian resources in wetlands and their effect on amphibian populations

Variation in algal and plant biomass and plant richness were not associated with differences in nutrient concentrations as predicted. However, algal biomass was significantly lower in restored wetlands. Interestingly, nitrate-N concentrations were higher, on average, in restored wetlands which may suggest that differences in algal biomass may be driven by other properties either associated with the restoration or not. For example, higher turbidity can reduce light attenuation and consequently algal biomass in wetlands (Truchy et al 2015). If hydrologic reconnection increased sediment inputs to restored wetlands, algal growth may have been inhibited. Furthermore, algal biomass can be controlled by dense stands of emergent vegetation that shade periphyton growth. Other studies have shown that algal primary production is lower when grazers (e.g. mollusks, mayflies, tadpoles) are present than when they are absent (Wallace et al 1996; Earl and Semlitsch 2012). If grazers were more abundant in restored wetlands, they may have reduced algal biomass. Therefore, variation in algal biomass may be attributed to reduced light attenuation or higher abundance of grazers in restored wetlands.

Although algal biomass was higher in restored wetlands than unrestored wetlands, it did not explain variation in amphibian abundance and biomass. This suggests that algal biomass is not a limiting resource to amphibians, despite algae being a primary food source for larval amphibians. The species collected during this study are generalist foragers (Hecnar 2004). For example, American bullfrog tadpoles primarily feed on algae and other aquatic plants but they will also forage on detrital material and consume decaying animal matter (Harding and Mifsud 2017). Therefore, while reconnection altered a primary amphibian resource, it is possible that other food sources subsidized lower algal biomass production in restored wetlands.

Total tadpole abundance and American bullfrog tadpole abundance and biomass declined with higher wetland plant biomass irrespective of wetland status, suggesting that dense stands of emergent vegetation generate poor habitat conditions. However, this effect on total tadpole abundance is likely driven by the relationship with American bullfrog tadpoles. When analyzed individually, abundance of the other amphibian species (i.e. all but American bullfrogs) was not associated with wetland plant. Tall emergent vegetation can reduce light attenuation and limit algal productivity and change algal composition. Perez et al. (2013) found that under high common reed densities, phytoplankton groups important to amphibian larval diet were less abundant. However, lower algal biomass in restored wetlands was not limiting to American bullfrog populations. Shading can also limit submerged wetland plant growth, which provides refuge and breeding and oviposition sites (Egan and Paton 2004). American bullfrog tadpoles may have experienced more predation if emergent plant biomass limited submerged aquatic habitat. Additionally, the structure of shoreline features (e.g. the shallows) may provide important nursery habitat (Hecnar 2004). Wetland vegetation along edges in both restored and unrestored wetlands were commonly populated with narrowleaf cattail and common reed. Furthermore, detrital material from decaying reeds and other invasive can produce toxins that limit larval growth and survival (Bucciarelli et al 2014). Perhaps dense stands of emergent vegetation reduced algal productivity and limited submerged aquatic habitat in the shallows, thus limiting key resources in an important habitat.

Adult amphibian abundance of all species heard calling varied seasonally following typical breeding patterns. American bullfrog abundance declined during the sampling period (Figure 2.4). American bullfrogs begin breeding mid-May, tapering off in late-July (Harding and Mifsud 2017). Green frog abundance peaked in July (Figure 2.6). Green frogs begin breeding mid-May and continue until August (Harding and Mifsud 2017). On the other hand, the increase in American toad and gray treefrog abundance over the sampling period (Figures 2.7, 2.8) was likely due to sampling error. American toad exhibit explosive breeding from late-April to late-May, while gray treefrog begin breeding in late-April and continue until July (Harding and Mifsud 2017).

Therefore, variation in adult amphibian abundance over time may be an effect of typical breeding patterns or sampling error.

Several species that were recorded within or near ONWR by the Great Lakes Marsh Monitoring Program were not detected during this study, suggesting that these wetlands are not suitable habitat for some amphibians. Permanent wetlands dominated by emergent vegetation are poor habitat for many sensitive amphibians. Micachion (2004) developed an amphibian index of biotic integrity for Ohio wetlands. He found a small range of possible scores for emergent wetlands, suggesting that communities do not vary enough across levels of disturbance to use them as indicators (Micacchion 2004). Additionally, many sensitive amphibians found in western Lake Erie basin wetlands, including Blanchard's cricket frog (*Acris crepitans blanchardi*) and wood frog (*Rana sylvatic*) have affinities for temporary wetlands in landscapes that are largely forested (Hecnar 2004) (Appendix C, Table C.1). Permanent wetlands favor more generalist species, including American bullfrog and green frog that require multiple years to metamorphose. Permanent, emergent wetlands also lack within-wetland habitat features favored by sensitive amphibian species. Primarily the absence of predatory fish. The presence of predatory fish, particularly non-native fish, in wetlands can result in low presence and diversity of amphibians (Hamer and McDonnell 2008). Common Carp (*Cyprinis carpio*) are present in ONWR and were found in traps set in both restored and unrestored wetlands. Several of the amphibians identified in this study can co-occur with predatory fish, including American bullfrog, green frog, and American toad because their eggs or larvae are unpalatable (Knutson et al 2004). The presence of these species in

ONWR wetlands likely reflects their ability to avoid fish predation. Additionally, the absence of more sensitive taxa could be due to the presence of predatory fish. Furthermore, some species that were not detected have shown decline in the Great Lakes basin. Chorus frogs (*Pseudacris sp.*) have experienced decline basin-wide (Tozer 2013). Wood frogs and spring peepers (*Acris crepitans blanchardi*) remained stable outside of AOC boundaries but experienced decline within AOCs (Tozer 2013). Generalists are more frequently found in wetlands in disturbed aquatic environments (Hamer and McDonnell 2008). For example, American toads are common in highly fragmented and human-modified environments (Houlahan and Findlay 2003). Therefore, wetlands in ONWR may not be suitable habitat for all amphibians historically detected in the county.

While species richness was low relative to other nearby wetlands and historical data collected in ONWR, the absence of particular species may be an effect of sampling effort (Herdendorf 1987; King et al 1997; Bird Studies Canada 2018) (Appendix C, Table C.1). Several species that were absent or detected at low abundance in this study begin breeding in April, including wood frog, chorus frogs, and northern leopard frog (Knutson et al 1999). These species have been detected using call surveys in and around ONWR as early as 1996. Therefore, the absence or low detection of these species may be an effect of sampling effort.

Field-oriented research presents conditions and circumstances beyond the control of researchers. Although unrestored wetlands were hydrologically isolated from Crane Creek and Lake Erie by earthen and rock dikes, management practices and natural processes facilitated some water exchange. Water levels in all wetland units are artificially managed with occasional drawdowns by pumping water in and out of the units depending on management goals. In 2017, one of the restored wetland units was drawn down to control for invasive wetland plants. Additionally, intense precipitation and storm events as well as seichal events caused dike overflow in unrestored wetlands in 2017 during the time of this study (Ron Huffman pers comm). TP concentrations in restored and unrestored wetlands may have been similar because of water level management and periodic water exchange between unrestored wetlands, Crane Creek, and Lake Erie.

4.4 Conclusion

Overall, properties of the restoration did not appear to affect amphibian abundance and biomass. Instead, amphibian populations reflected properties of ONWR irrespective of wetland status. Although hydrologic reconnection may have led to higher nitrate-N in restored wetlands, this did not appear to increase nitrate-N levels above concentrations that would directly affect amphibians. Furthermore, although algal biomass was lower in restored wetlands and is a primary larval resource, it did not explain variation in amphibian tadpole abundance and biomass. American bullfrog abundance and biomass were negatively affected by higher plant biomass irrespective of wetland status, suggesting that American bullfrog populations may by influenced by dense stands of emergent vegetation that are characteristic of wetland edges throughout ONWR. Other features of wetlands in ONWR such as water permanence, the presence of predatory fish, and location within the Maumee AOC likely exclude some amphibians that were historically detected in the county. While hydrologically reconnecting wetlands has the potential to limit habitat fragmentation and increase habitat availability, restored wetlands in ONWR are still largely bound by dikes and managed to maintain permanent water. Effective amphibian conservation efforts will likely require a combination of permanent and temporary wetlands that cater to less robust, specialist amphibian populations. To better understand responses of amphibian abundance and biomass in the Maumee AOC to hydrologic restoration, a more diverse set of wetlands including temporary, non-emergent, and forested wetlands should be examined

Tables

Table 2. 1 Linear mixed effect models where nitrate-N and TP were dependent variables, status, time, and status*time were fixed effects, and site was a random effect. Significance codes: * = 0.05.

	Status		**Time**		**Status:Time**			
	T	***P***	**T**	***P***	**T**	***P***	**R^2m**	**R^2c**
Nitrate-N	**-3.01**	**0.004***	**-6.82**	**<0.0001***	**3.28**	**0.002***	0.33	0.46
TP	-0.28	0.78	-0.68	0.50	0.51	0.61	0.004	0.27

Table 2. 2 Linear mixed effects models where AFDM was the dependent variable, status and nitrate-N or TP were fixed effects, and site was a random effect. Significance codes: * = 0.05.

AFDM

Nitrate-N (*R^2m=0.12, R^2c=0.27)*

	T	***P***
Status	**2.32**	**0.04***
Nitrate-N	-0.41	0.68

TP (*R^2m=0.15, R^2c=0.31)*

	T	***P***
Status	**2.26**	**0.047***
TP	1.71	0.09

Table 2. 3 Linear models where plant richness and plant biomass were dependent variables and A) nitrate-N or B) TP was the fixed effect. Significance codes: * = 0.05.

A)

	Status	**Nitrate-N**	
	P	***P***	**R^2**
Plant richness	0.07	0.91	0.33
Plant biomass	0.70	0.30	0.21

B)

	Status	**TP**	
	P	***P***	**R^2**
Plant richness	0.17	0.17	0.47
Plant biomass	0.68	0.77	0.05

Table 2. 4 Linear mixed effects models where tadpole metrics were the dependent variables, status was the fixed effect, and site was the random effect. Significance codes: * = 0.05.

	Status			
	T	***P***	**R^2m**	**R^2c**
Total tadpole CPUE	0.22	0.83	0.002	0.38
American bullfrog tadpole CPUE	0.71	0.50	0.02	0.10
Northern leopard frog tadpole CPUE	-1.27	0.21	0.04	0.04
Green frog tadpole CPUE	0.69	0.49	0.01	0.01
American bullfrog tadpole biomass	1.11	0.29	0.06	0.38
Northern leopard frog tadpole biomass	-1.22	0.23	0.04	0.04
Green frog tadpole biomass	0.76	0.45	0.02	0.02

Table 2. 5 Linear mixed effects models where call codes of American bullfrog, green frog, American toad, and gray treefrog were the dependent variables, status, time, and status*time were fixed effects, and survey nested within site was a random effect. Significance codes: * = 0.05.

	Status		**Time**		**Status:Time**			
	T	***P***	**T**	***P***	**T**	***P***	**R^2m**	**R^2c**
American bullfrog call code	1.50	0.14	**-4.91**	**<0.0001***	-1.44	0.16	0.43	0.60
Green frog call code	**2.87**	**0.01***	-0.47	0.64	**-2.06**	**0.04***	0.22	0.34
American toad call code	1.92	0.06	**4.49**	**<0.0001***	**-2.53**	**0.01***	0.20	0.32
Gray treefrog call code	0.87	0.39	**4.06**	**<0.0001***	-1.48	0.14	0.17	0.43

Table 2. 6 Linear mixed effects models where tadpole metrics were dependent variables, status and nitrate-N were fixed effects and site was a random effect. Significance codes: * = 0.05.

	Status		**Nitrate-N**			
	T	***P***	**T**	***P***	**R^2m**	**R^2c**
Total tadpole CPUE	0.18	0.86	-0.50	0.62	0.01	0.37
American bullfrog tadpole CPUE	0.65	0.53	-0.53	0.60	0.02	0.09
Northern leopard frog tadpole CPUE	-1.06	0.32	1.96	0.06	0.14	0.15
Green frog tadpole CPUE	0.68	0.50	0.05	0.96	0.01	0.01
American bullfrog tadpole biomass	1.07	0.31	-0.52	0.61	0.06	0.37
Northern leopard frog tadpole biomass	-1.01	0.34	2.08	0.06	0.14	0.16
Green frog tadpole biomass	0.76	0.46	0.10	0.92	0.02	0.02

Table 2. 7 Linear mixed effects models where tadpole metrics were dependent variables, status and AFDM were fixed effects and site was a random effect. Significance codes: * = 0.05.

	Status		**AFDM**			
	T	***P***	**T**	***P***	**R^2m**	**R^2c**
Total tadpole CPUE	0.10	0.92	0.56	0.58	0.01	0.38
American bullfrog tadpole CPUE	0.67	0.51	-0.14	0.89	0.02	0.08
Northern leopard frog tadpole CPUE	-1.58	0.14	1.30	0.21	0.08	0.09
Green frog tadpole CPUE	1.37	0.18	-1.57	0.13	0.08	0.08
American bullfrog tadpole biomass	1.34	0.20	-0.79	0.44	0.07	0.36
Northern leopard frog tadpole biomass	-1.59	0.14	1.32	0.20	0.08	0.10
Green frog tadpole biomass	1.46	0.17	-1.65	0.11	0.09	0.10

Table 2. 8 Linear models where amphibian metrics were dependent variables, status and plant richness was the fixed effect. Significance codes: * = 0.05.

	Status	**Plant richness**	
	P	***P***	**R^2**
Total tadpole CPUE	0.32	0.06	0.35
American bullfrog tadpole CPUE	0.73	0.12	0.28
Northern leopard frog tadpole CPUE	0.09	0.21	0.29
Green frog tadpole CPUE	0.58	0.97	0.05
American bullfrog call code	0.96	0.18	0.26
Green frog call code	0.42	0.054	0.56
American toad call code	0.20	0.30	0.18
Gray treefrog call code	0.42	0.63	0.07
American bullfrog tadpole biomass	0.51	0.76	0.12
Northern leopard frog tadpole biomass	0.11	0.25	0.26
Green frog tadpole biomass	0.11	0.25	0.26

Table 2. 9 Linear models where amphibian metrics were dependent variables, status and plant biomass was the fixed effect. Significance codes: * = 0.05.

	Status	**Plant biomass**	
	P	***P***	**R^2**
Total tadpole CPUE	0.45	**0.03***	0.42
American bullfrog tadpole CPUE	0.17	**0.02***	0.51
Northern leopard frog tadpole CPUE	0.31	0.20	0.30
Green frog tadpole CPUE	0.28	0.10	0.31
American bullfrog call code	0.26	0.36	0.18
Green frog call code	0.08	0.16	0.47
American toad call code	0.48	0.64	0.10
Gray treefrog call code	0.69	0.15	0.25
American bullfrog tadpole biomass	0.08	**0.02***	0.53
Northern leopard frog tadpole biomass	0.33	0.22	0.28
Green frog tadpole biomass	0.25	0.09	0.33

Figures

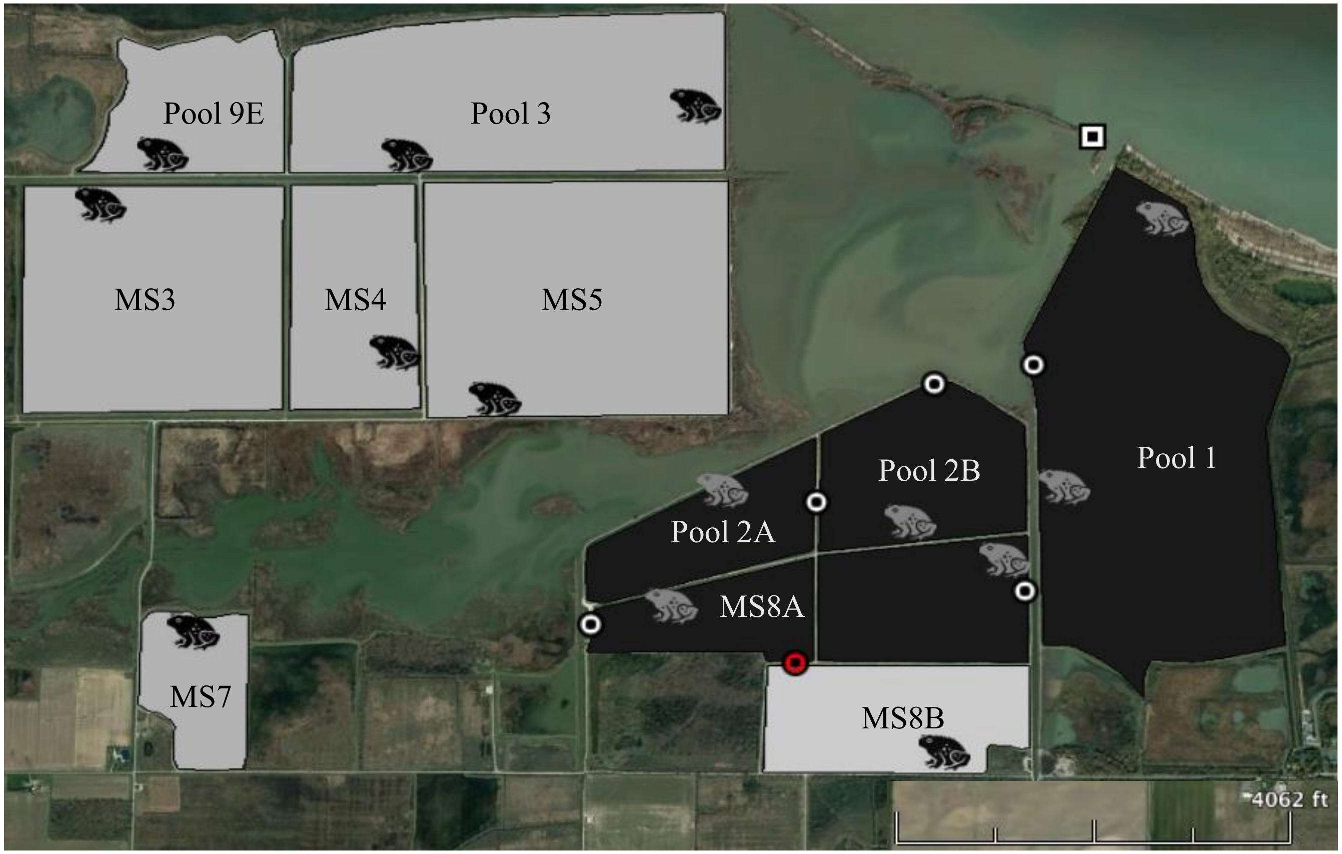

Figure 2. 1 Ottawa National Wildlife Refuge complex. Unrestored wetlands indicated by gray polygons, restored wetlands indicated by black polygons. Active water control structures indicated by white circles, inactive water control structure indicated by red circle. Opening to Lake Erie indicated by white square. Call survey and trap locations indicated by amphibian icon.

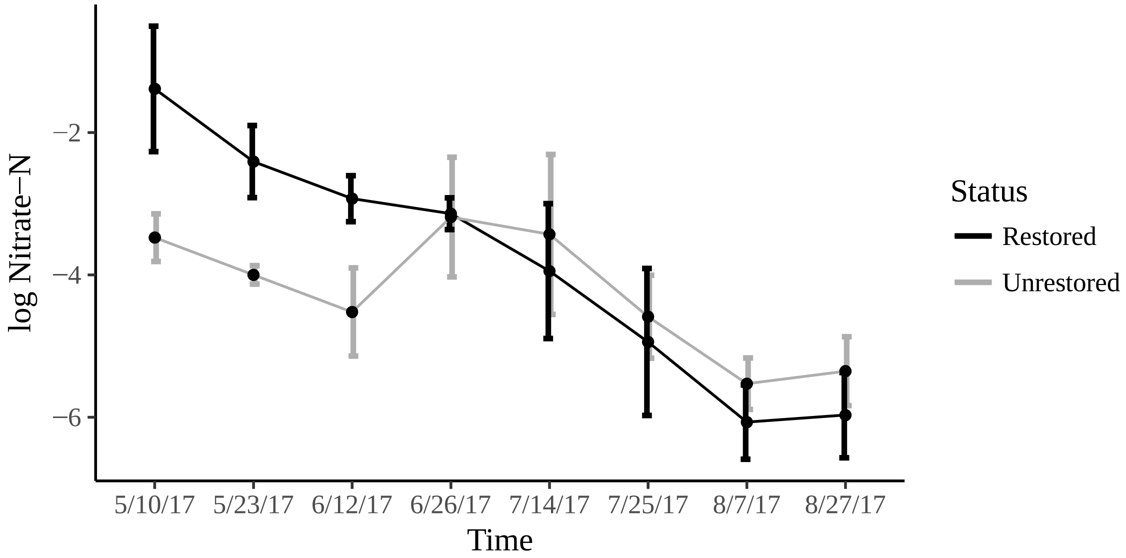

Figure 2. 2 Log-transformed nitrate-N in restored and unrestored wetlands over time.

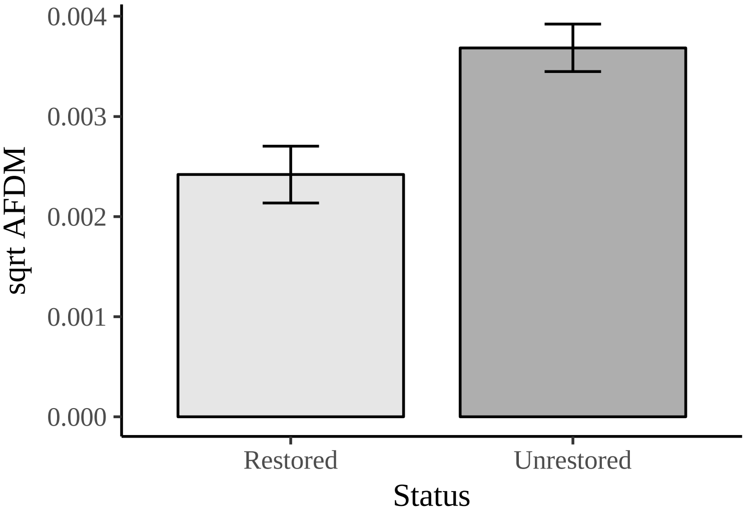

Figure 2. 3 Square root transformed AFDM in restored and unrestored wetlands.

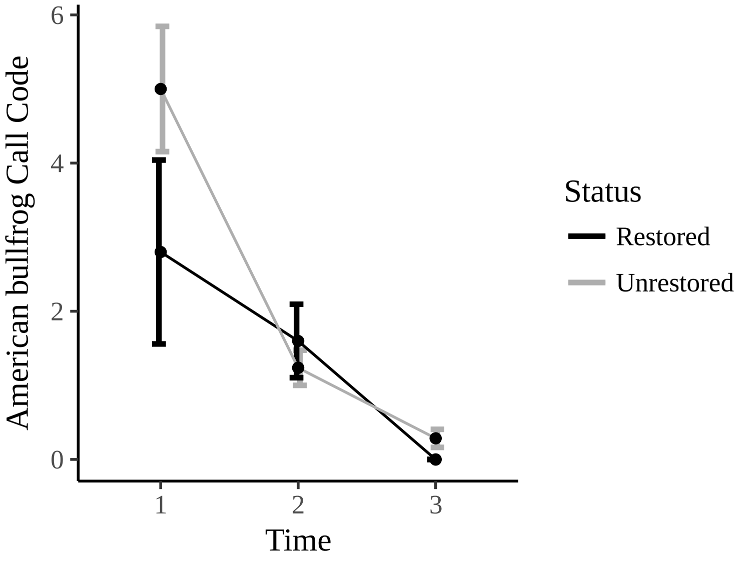

Figure 2. 4 Mean and standard error of American bullfrog call code in restored and unrestored wetlands over time.

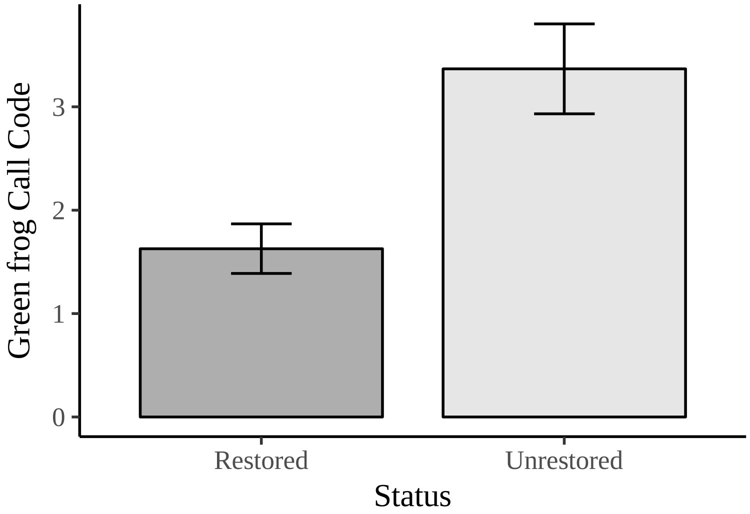

Figure 2. 5 Mean and standard error of green frog call code in restored and unrestored wetlands.

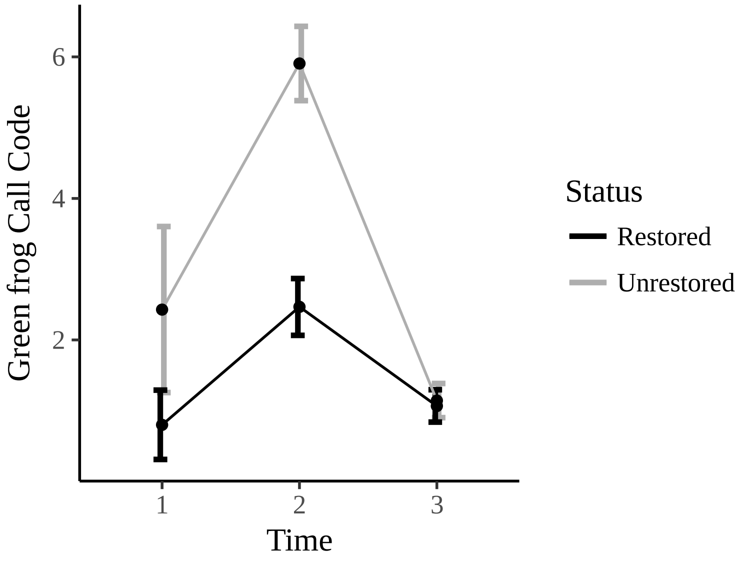

Figure 2. 6 Mean and standard error of green frog call code in restored and unrestored wetlands over time.

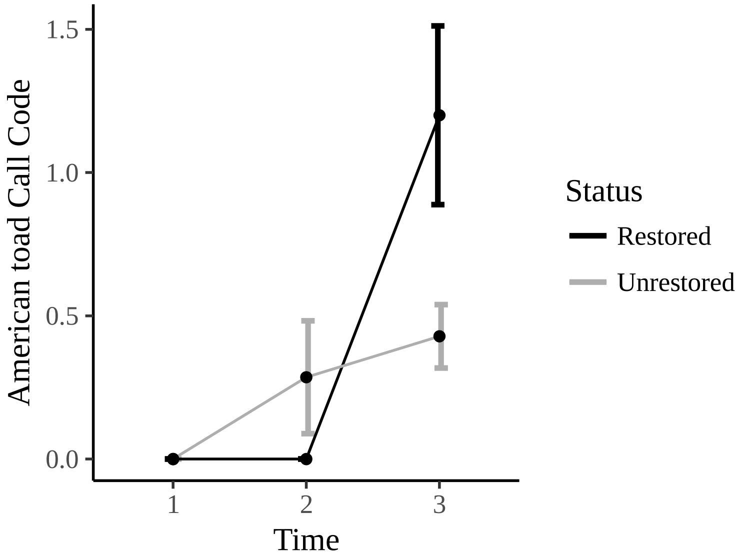

Figure 2. 7 Mean and standard error of American toad call code in restored and unrestored wetlands over time.

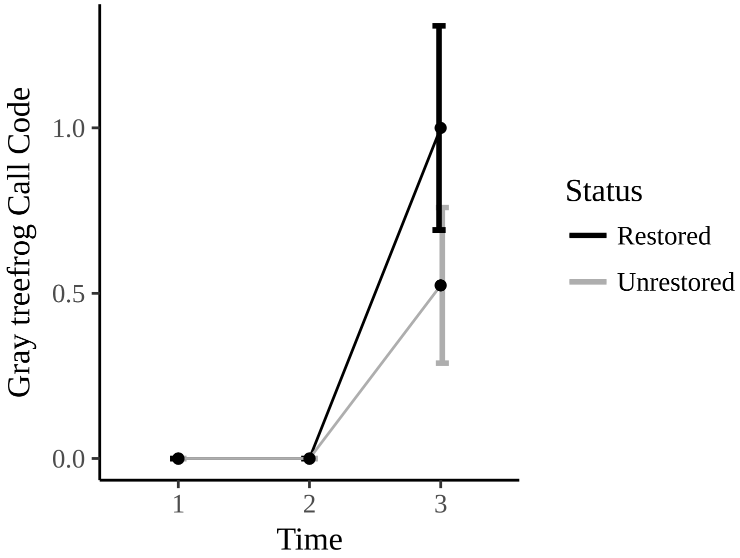

Figure 2. 8 Mean and standard error of gray treefrog call code in restored and unrestored wetlands over time.

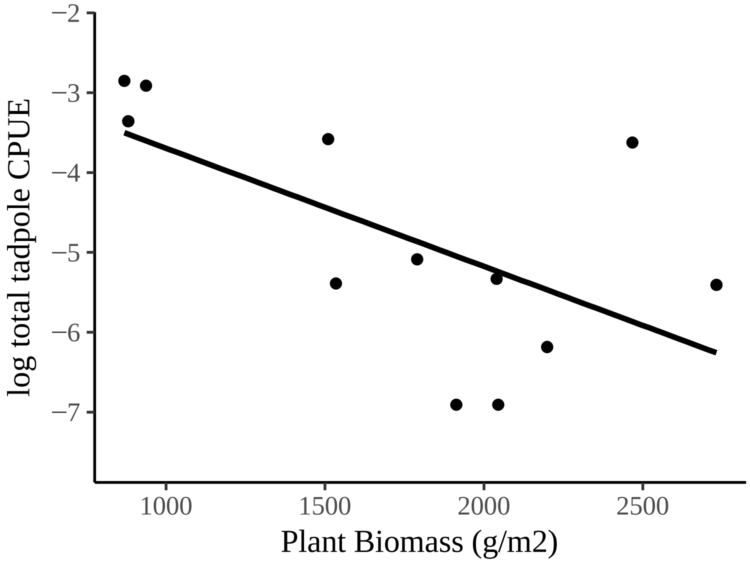

Figure 2. 9 Log transformed total tadpole CPUE and plant biomass.

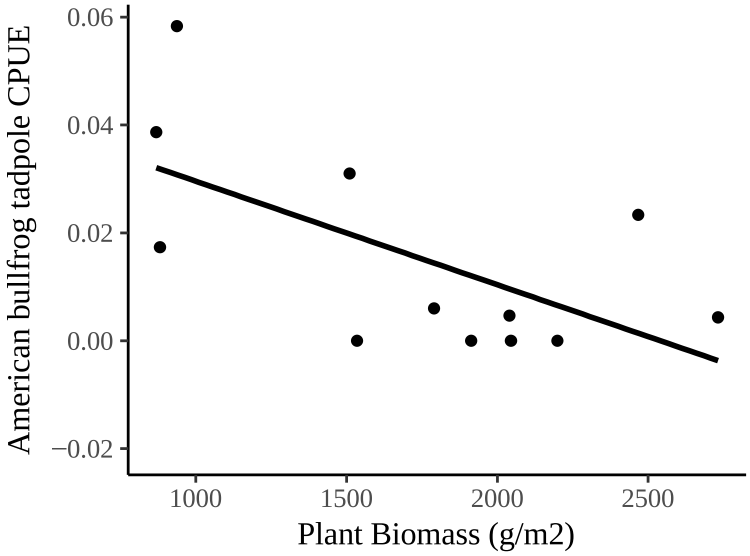

Figure 2. 10 American bullfrog tadpole CPUE and plant biomass.

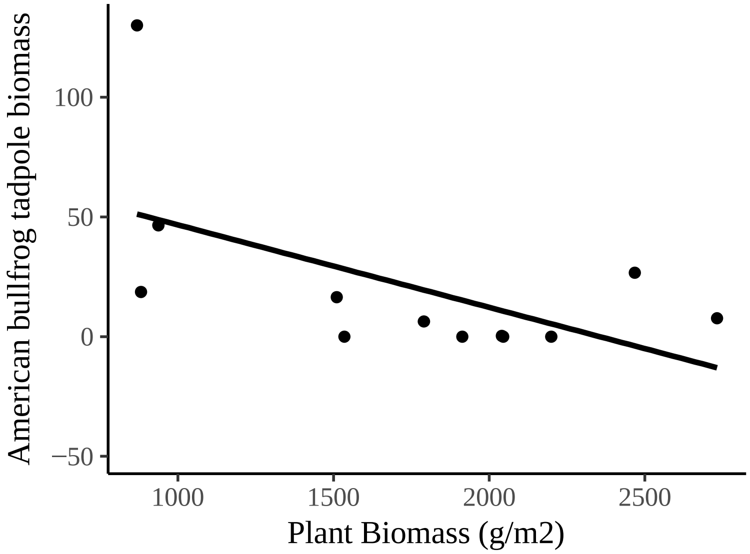

Figure 2. 11 American bullfrog tadpole biomass and plant biomass.

References

Ashley PE, Robinson JT (1996) Road Mortality of Amphibians, Reptiles and Other Wildlife on the Long Point Causeway, Lake Erie, Ontario. The Canadian Field Naturalist 110:403–412.

Baker JMR, Waights V (1994) The effects of nitrate on tadpoles of the tree frog (Litoria caerulea). Herpetological Journal 4:106–108.

Barrett K, Crawford JA, Milanovich JR (2017) Detritus Quality Produces Species-Specific Tadpole Growth and Survivorship Responses in Experimental Wetlands Aquatic Insect Response to Development in Southern Appalachia View project Landscape-Scale Conservation Planning View project. Article in Journal of Herpetology. doi: 10.1670/16-091

Bates D, Mächler M, Bolker B, Walker S (2014) Fitting Linear Mixed-Effects Models using lme4. doi: 10.18637/jss.v067.i01

Batzer DP (1996) Ecology of Insect Communities in Nontidal Wetlands. Annual Review of Entomology 41:75–100. doi: 10.1146/annurev.ento.41.1.75

Bird Studies Canada (2018) Marsh Monitoring Program. In: NatureCounts, a node of the Avian Knowledge Network, Bird Studies Canada. http://www.naturecounts.ca/. Accessed 13 Nov 2018

Bishop CA, Mahony NA, Struger J, et al (1999) Anuran development, density and diversity in relation to agricultural activity in the Holland River Watershed, Ontario, Canada (1990-1992).

Blanco MA, Whitten WM, Williams NH (2006) A Simple and Safe Method for Rapid Drying of Plant Specimens Using Forced-Air Space Heaters Estudios de Huilaea y Chalybea (Melastomataceae) View project Phylogenetic and taxonomic studies on Polygalaceae View project. doi: 10.2307/41760265

Blann KL, Anderson JL, Sands GR, Vondracek B (2009) Effects of agricultural drainage on aquatic ecosystems: a review. Critical Reviews in Environmental Science and Technology 39:909–1001.

Borja Á, Dauer DM, Elliott M, Simenstad CA (2010) Medium-and Long-term Recovery of Estuarine and Coastal Ecosystems: Patterns, Rates and Restoration Effectiveness. Estuaries and Coasts 33:1249–1260. doi: 10.1007/s12237-010-9347-5

Bucciarelli GM, Blaustein AR, Garcia TS, Kats LB (2014) Invasion Complexities: The Diverse Impacts of Nonnative Species on Amphibians. Source: Copeia 14:611–632. doi: 10.1643/OT-14-014

Burne MR, Griffin CR (2005) Habitat associations of pool-breeding amphibians in eastern Massachusetts, USA. Wetlands Ecology and Management 13:247–259. doi: 10.1007/s11273-004-7519-4

Cadotte MW (2011) Beyond species : functional diversity and the maintenance of ecological processes and services . J Appl Ecol Beyond species : functional diversity and the maintenance of ecological processes and services. Journal of Applied Ecology 48:1079–1087. doi: 10.1111/j.1365-2664.2011.02048.x

Chambers RM, Meyerson L a., Saltonstall K (1999) Expansion of *Phragmites australis* into tidal wetlands of North America. Aquatic Botany 64:261–273. doi: 10.1016/S0304-3770(99)00055-8

Cooper MJ, Uzarski DG, Burton TM (2007) Macroinvertebrate community composition in relation to anthropogenic disturbance, vegetation, and organic sediment depth in four lake michigan drowned river-mouth wetlands. Wetlands 27:894–903.

Cosentino BJ, Marsh DM, Jones KS, et al (2014) Citizen science reveals widespread negative effects of roads on amphibian distributions. doi: 10.1016/j.biocon.2014.09.027

Cummins KW, Merritt RW, Berg MB (1996) An introduction to the aquatic insects of North America. Kendall.

Díaz AM, Alonso MLS, Gutiérrez MRVA (2008) Biological traits of stream macroinvertebrates from a semi-arid catchment: Patterns along complex environmental gradients. Freshwater Biology 53:1–21. doi: 10.1111/j.1365-2427.2007.01854.x

Dodds WK, Smith VH, Lohman K (2002) Nitrogen and phosphorus relationships to benthic algal biomass in temperate streams. doi: 10.1139/F02-063

Earl JE, Semlitsch RD (2012) Reciprocal subsidies in ponds: Does leaf input increase frog biomass export? Oecologia 170:1077–1087. doi: 10.1007/s00442-012-2361-5

Egan RS, Paton PWC (2004) Within-pond parameters affecting oviposition by wood frogs and spotted salamanders.

Greenberg DA, Green DM (2013) Effects of an invasive plant on population dynamics in toads. Conservation Biology 27:1049–1057. doi: 10.1111/cobi.12078

Hamer AJ, McDonnell MJ (2008) Amphibian ecology and conservation in the urbanising world: A review. Biological Conservation 141:2432–2449. doi: 10.1016/j.biocon.2008.07.020

Harding JH, Mifsud DA (2017) Amphibians and reptiles of the Great Lakes region. University of Michigan Press

Hecnar SJ (2004) Great Lakes wetlands as amphibian habitats: A review. Aquatic Ecosystem Health & Management 7:289–303. doi: 10.1080/14634980490461542

Hecnar SJ (1995) Acute and chronic toxicity of ammonium nitrate fertilizer to amphibians from southern ontario. Environmental Toxicology and Chemistry 14:2131–2137. doi: 10.1002/etc.5620141217

Heino J (2008) Patterns of functional and function-environment relationships biodiversity in lake littoral macroinvertebrates. Limnology and Oceanography 53:1446–1455. doi: 10.2307/40058265

Herdendorf CE (1987) The Ecology of the Coastal Marshes of Western Lake Erie: A Community Profile. U.S. Fish and Wildlife Service Biology Report 85:171.

Holomuzki JR, Klarer DM (2010) Invasive reed effects on benthic community structure in Lake Erie coastal marshes. Wetlands Ecology and Management 18:219–231. doi: 10.1007/s11273-009-9161-7

Houlahan JE, Findlay CS (2003) The effects of adjacent land use on wetland amphibian species richness and community composition. Canadian Journal of Fisheries and Aquatic Sciences 60:1078–1094. doi: 10.1139/f03-095

International Joint Commission United States and Canada (1987) Protocol Amending the 1978 Agreement Between the United States of America and Canada on Great Lakes Water Quality as Amended on October 16, 1983.

Jordan TE, Whigham DF, Hofmockel KH, Pittek M a (2000) Nutrient and sediment removal by a restored wetland receiving agricultural runoff. Journal of Environmental Quality 32:1534–1547. doi: 10.2134/jeq2003.1534

Julie KC, Fennessy MS (2001) Wetland plants: biology and ecology.

King RB, Oldham MJ, Weller WF, Wynn D (1997) Historic and Current Amphibian and Reptile Distributions in the Island Region of Western.

King RS, Richardson CJ (2007) Subsidy–stress response of macroinvertebrate community biomass to a phosphorus gradient in an oligotrophic wetland ecosystem. Journal of the North American Benthological Society. doi: 10.1899/06-002R.1

Knutson MG, Richardson WB, Reineke DM, et al (2004) Agricultural ponds support amphibian populations. Ecological Applications 14:669–684. doi: 10.1890/02-5305

Knutson MG, Sauer JR, Olsen DA, et al (1999) Effects of landscape composition and wetland fragmentation on amphibian diversity. Conservation Biology 13:1437–1446.

Kowalski KP, Wiley MJ, Wilcox DA, et al (2014a) 2011 Summary: Coastal Wetland Restoration Research.

Kowalski KP, Wiley MJ, Wilcox DA (2014b) Fish Assemblages, Connectivity, and Habitat Rehabilitation in a Diked Great Lakes Coastal Wetland Complex. Transactions of the American Fisheries Society 143:1130–1142. doi:

10.1080/00028487.2014.911207

Kuznetsova A, Brockhoff PB, Christensen RHB (2017) **lmerTest** Package: Tests in Linear Mixed Effects Models. Journal of Statistical Software. doi: 10.18637/jss.v082.i13

Lake Erie Lakewide Management Plan Work Group (2011) Lake Erie Binational Nutrient Managment Strategy.

Laliberté E, Legendre P, Bill Shipley (2015) Measuring functional diversity (FD) from multiple traits, and other tools for functional ecology. 1–28. doi: http://cran.r-project.org/web/packages/FD/FD.pdf

Ledger ME, Edwards FK, Brown LE, et al (2011) Impact of simulated drought on ecosystem biomass production: An experimental test in stream mesocosms. Global Change Biology 17:2288–2297. doi: 10.1111/j.1365-2486.2011.02420.x

Lougheed VL, Crosbie B, Chow-Fraser P (2001) Primary determinants of macrophyte community structure in 62 marshes across the Great Lakes basin: latitude, land use, and water quality effects. Canadian Journal of Fisheries and Aquatic Sciences 58:1603–1612. doi: 10.1139/f01-102

Maynard L, Wilcox DA (1997) Coastal Wetlands. State of the Lakes Ecosystem Conference. pp 1–103

McCormick P V, Shuford Iii RBE, Rawlik PS (2004) Changes in macroinvertebrate community structure and function along a phosphorus gradient in the Florida Everglades. Hydrobiologia 529:113–132.

Merritt RW, Cummins KW (2006) Trophic Relationships of Macroinvertebrates based on feeding, Second Edi. Methods in Stream Ecology. doi: 10.1016/B978-012332908-0.50036-X

Meyer CK, Whiles MR (2008) Macroinvertebrate communities in restored and natural Platte River slough wetlands. Journal of the North American Benthological Society 27:626–639. doi: 10.1899/07-086.1

Micacchion M (2004) Integrated wetland assessment program. part 7: amphibian index of biotic integrity (AmphIBI) for Ohio wetlands. Ohio EPA, Wetland Ecology Group, Division of Surface … 31 pp.

Michalak AM, Anderson EJ, Beletsky D, et al (2013) Record-setting algal bloom in Lake Erie caused by agricultural and meteorological trends consistent with expected future conditions. Proceedings of the National Academy of Sciences 110:6448–6452. doi: 10.1073/pnas.1216006110

Mitsch WJ, Reeder BC, Klarer DM (1989) The role of wetlands in the control of nutrients with a case study of western Lake Erie. In: Mitsch WJ, Jorgensen SE (eds) Ecological Engineering: an Introduction to Ecotechnology. John Wiley, pp 129–158

Mitsch WJ, Wang NM (2000) Large-scale coastal wetland restoration on the Laurentian Great Lakes: Determining the potential for water quality improvement. Ecological Engineering 15:267–282. doi: http://dx.doi.org/10.1016/S0925-8574(00)00081-1

Moreno-Mateos D, Power ME, Comín FA, Yockteng R (2012) Structural and functional loss in restored wetland ecosystems. PLoS Biology. doi: 10.1371/journal.pbio.1001247

Niemi GJ, Brady VJ, Brown TN, et al (2009) Development of ecological indicators for the US Great Lakes coastal region - A summary of applications in Lake Huron. Aquat Ecosyst Health 12:77–89. doi: 10.1080/14634980802711943

NOAA (2018) HAB Bulletin. https://www.glerl.noaa.gov/res/HABs_and_Hypoxia/bulletin.html. Accessed 15 Oct 2018

Ohio EPA (2018) Nutrient Mass Balance Study for Ohio’s Major Rivers.

Ohio EPA (2012) Total Maximum Daily Loads for the Maumee River (lower) Tributaries and Lake Erie Tributaries Watershed.

Palmer MA, Menninger HL, Bernhardt E (2010) River restoration, habitat heterogeneity and biodiversity: A failure of theory or practice? Freshwater Biology 55:205–222. doi: 10.1111/j.1365-2427.2009.02372.x

Pearl CA, Adams MJ, Leuthold N, Bruce Bury R (2005) Amphibian occurence and aquatic invaders in a changing landscape: implications for wetland mitigation in the Willamette Valley, Oregon, USA. Wetlands 25:76–88.

Perez A, Mazerolle MJ, Brisson J (2013) Effects of exotic common reed (Phragmites australis) on wood frog (Lithobates sylvaticus) tadpole development and food availability. doi: 10.1080/02705060.2012.750629

Price SJ, Howe RW, Hanowski JM, et al (2007) Are Anurans of Great Lakes Coastal Wetlands Reliable Indicators of Ecological Condition Are Anurans of Great Lakes Coastal Wetlands Reliable Indicators of Ecological Condition ? 33:211–223.

Rader RB, Richardson CJ (1994) Response of macroinvertebrates and small fish to nutrient enrichment in the northern Everglades. Wetlands 14:134–146. doi:

10.1007/BF03160629

Raichel DL, Able KW, Hartman JM (2003) The influence of Phragmites (common reed) on the distribution, abundance, and potential prey of a resident marsh fish in the Hackensack Meadowlands, New Jersey. Estuaries 26:511–521. doi: 10.1007/BF02823727

Rao CR (1982) Diversity and dissimilarity coefficients—a unified approach. Theoretical Population Biology 21:24–43.

Redfield AC, Ketchum BH, Richards FA (1963) The influence of organisms on the composition of sea-water.

Rouse JD, Bishop CA, Struger J (1999) Nitrogen pollution: An assessment of its threat to amphibian survival. Environmental Health Perspectives 107:799–803. doi: 10.1289/ehp.99107799

Saunders DL, Kalff J (2001) Nitrogen rentention in wetlands, lakes and rivers. Hydrobiologia 443:205–212.

Schmera D, Heino J, Podani J, et al (2017) Functional diversity: a review of methodology and current knowledge in freshwater macroinvertebrate research. Hydrobiologia 787:27–44. doi: 10.1007/s10750-016-2974-5

Schock NT, Murry BA, Uzarski DG (2014) Impacts of Agricultural Drainage Outlets on Great Lakes Coastal Wetlands. Wetlands 34:297–307. doi: 10.1007/s13157-013-0486-x

Scott JT, Doyle RD, Filstrup CT (2005) Periphyton nutrient limitation and nitrogen fixation potential along a wetland nutrient-depletion gradient. Wetlands 25:439–448.

doi: 10.1672/18

Semlitsch RD (2000) Principles for Management of Aquatic-Breeding Amphibians. Journal of Wildlife Management 64:615–631.

Sierszen ME, Morrice JA, Moffett MF, West CW (2004) Benthic Versus Planktonic Foundations of Three Lake Superior Coastal Wetland Food Webs. Journal of Great Lakes Research. doi: 10.1016/S0380-1330(04)70327-5

Sierszen ME, Morrice JA, Trebitz AS, Hoffman JC (2012) A review of selected ecosystem services provided by coastal wetlands of the Laurentian Great Lakes. Aquatic Ecosystem Health & Management 15:92–106. doi: 10.1080/14634988.2011.624970

Smith JP (2017) North American Floras, Manuals, and Checklist: A Bibliography.

Stephens JP, Berven KA, Tiegs SD (2013) Anthropogenic changes to leaf litter input affect the fitness of a larval amphibian. Freshwater Biology 58:1631–1646. doi: 10.1111/fwb.12155

Sullivan PL, Gaiser EE, Surratt D, et al (2014) Wetland ecosystem response to hydrologic restoration and management: The everglades and its urban-Agricultural boundary (FL, USA). Wetlands. doi: 10.1007/s13157-014-0525-2

Surratt D, Shinde D, Aumen N (2012) Recent cattail expansion and possible relationships to water management: Changes in upper taylor slough (Everglades National Park, Florida, USA). Environmental Management 49:720–733. doi: 10.1007/s00267-011-9798-x

Tozer DC (2013) The Great Lakes Marsh Monitoring Program unique survey locations .

Truchy A, Angeler DG, Sponseller RA, et al (2015) Linking biodiversity, ecosystem functioning and services, and ecological resilience: Towards an integrative framework for improved management. Advances in Ecological Research. pp 55–96

Uzarski DG, Brady VJ, Cooper MJ, et al (2017) Standardized Measures of Coastal Wetland Condition: Implementation at a Laurentian Great Lakes Basin-Wide Scale. Wetlands 37:15–32. doi: 10.1007/s13157-016-0835-7

Uzarski DG, Brady VJ, Cooper MJ (2015) Quality Assurance Project Plan GLIC : Implementing Great Lakes Coastal Wetland Monitoring. 1–98.

Villéger S, Mason H, Mouillot D (2008) New Multidimensional Functional Diversity Indices for a Multifaceted Framework in Functional Ecology Author (s): Sébastien Villéger , Norman W . H . Mason , David Mouillot Published by : Ecological Society of America content in a trusted digital archive. Ecology 89:2290–2301. doi: 10.1890/07-1206.1

Voss EG, Reznicek AA (2012) Field manual of Michigan flora. University of Michigan Press

Wallace JB, Webster JR, Meyer JL, et al (1996) The role of macroinvertebrates in stream ecosystem function. Annual review of entomology 41:115–39. doi: 10.1146/annurev.en.41.010196.000555

Wang N, Mitsch WJ (1998) Estimating phosphorus retention of existing and restored coastal wetlands in a tributary watershed of the Laurentian Great Lakes in Michigan, USA. Wetlands Ecology and Management 6:69–82. doi: 10.1023/A:1008451823394

Watson SB, Miller C, Arhonditsis G, et al (2016) The re-eutrophication of Lake Erie:

Harmful algal blooms and hypoxia. Harmful Algae 56:44–66. doi: 10.1016/j.hal.2016.04.010

Wolf KL, Noe GB, Ahn C (2013) Hydrologic Connectivity to Streams Increases Nitrogen and Phosphorus Inputs and Cycling in Soils of Created and Natural Floodplain Wetlands. Journal of Environmental Quality 42:1245–1255. doi: 10.2134/jeq2012.0466

Woltemade CJ (2000) Ability of Restored Wetlands to Reduce Nitrogen and Phosphorus Concentrations in Agricultural Drainage Water. Journal of Soil and Water Conservation 55:303–309.

Woodward G (2009) Biodiversity, ecosystem functioning and food webs in fresh waters: Assembling the jigsaw puzzle. Freshwater Biology 54:2171–2187. doi: 10.1111/j.1365-2427.2008.02081.x

Zedler JB, Kercher S (2005) Wetland Resources: Status, Trends, Ecosystem Services, and Restorability. Annual Review of Environment and Resources 30:39–74. doi: 10.1146/annurev.energy.30.050504.144248

Appendix A. Summary of macroinvertebrates

Table A. 1 Functional feeding group: filterer-collector (FC), gatherer-collector (GC), predator (PR), piercer-herbivore (PI), shredder (SH), scraper (SC); modes of existence (MOE): burrower (BU), climber (CB), clinger (CN), skater (SK), sprawler (SP), swimmer (SW); body size: small, medium, large; and voltinism: semivoltine, univoltine, and bi-multivoltine listed for each operational taxonomic unit (OTU).

Phylum	**Class**	**Order**	**Family**	**Genus**	**Species**	**OTU**	**FFG**	**MOE**	**Body Size**	**Voltinism**
Annelida	Clitellata					Hirudinea (subclass)	PR	SP	Large	NA
Annelida	Clitellata	Arhynchobdellida	Erpobdellidae	Erpobdella		Erpobdella	PR	SW	Large	NA
Annelida	Clitellata	Arhynchobdellida	Erpobdellidae	Erpobdella	punctata	Erpobdella punctata	PR	SW	Large	Semivoltine
Annelida	Clitellata	Rhynchobdellida	Glossiphoniidae			Glossiphoniidae	PR	CB	Large	NA
Annelida	Clitellata	Rhynchobdellida	Glossiphoniidae	Placobdella		Placobdella	PR	SW	Large	NA
Annelida	Clitellata	Rhynchobdellida	Glossiphoniidae	Placobdella	papillifera	Placobdella papillifera	PR	SW	Large	Univoltine
Annelida	Clitellata	Rhynchobdellida	Glossiphoniidae	Alboglossiphonia	heteroclita	Alboglossiphonia heteroclita	PR	SW	Small	Univoltine
Annelida	Clitellata	Rhynchobdellida	Glossiphoniidae	Helobdella		Helobdella	PR	SP	Medium	Bi-Multivoltine
Annelida	Clitellata	Rhynchobdellida	Glossiphoniidae	Helobdella	fusca	Helobdella fusca	PR	SP	Medium	Bi-Multivoltine
Annelida	Clitellata	Rhynchobdellida	Glossiphoniidae	Helobdella	papillata	Helobdella papillata	PR	SP	Medium	Bi-Multivoltine
Annelida	Clitellata	Rhynchobdellida	Glossiphoniidae	Helobdella	stagnalis	Helobdella stagnalis	PR	SP	Medium	Bi-Multivoltine
Annelida	Clitellata	Tubificida	Naididae			Naididae	GC	BU	Large	NA
Annelida	Clitellata	Tubificida	Naididae			Naidinae	GC	BU	Large	NA
Annelida	Clitellata	Tubificida	Naididae	Chaetogaster	diastrophus	Chaetogaster diastrophus	PR	BU	Medium	Bi-Multivoltine
Annelida	Clitellata	Tubificida	Naididae	Chaetogaster	limnaei	Chaetogaster limnaei	PR	BU	Medium	Bi-Multivoltine

Annelida	Clitellata	Tubificida	Naididae	Dero	digitata	Dero digitata	GC	BU	Large	Bi-Multivoltine
Annelida	Clitellata	Tubificida	Naididae	Dero	nivea	Dero nivea	GC	BU	Large	Bi-Multivoltine
Annelida	Clitellata	Tubificida	Naididae	Dero	vaga	Dero vaga	GC	BU	Large	Bi-Multivoltine
Annelida	Clitellata	Tubificida	Naididae	Nais		Nais	GC	BU	Medium	Bi-Multivoltine
Annelida	Clitellata	Tubificida	Naididae	Nais	communis/ variabilis complex	Nais communis/variabilis complex	GC	BU	Medium	Bi-Multivoltine
Annelida	Clitellata	Tubificida	Naididae	Slavina	appendiculata	Slavina appendiculata	GC	BU	Large	Bi-Multivoltine
Annelida	Clitellata	Tubificida	Naididae	Stylaria	lacustris	Stylaria lacustris	GC	BU	Large	Bi-Multivoltine
Annelida	Clitellata	Tubificida	Naididae	Pristina	longiseta	Pristina longiseta	GC	BU	Medium	Bi-Multivoltine
Annelida	Clitellata	Tubificida	Naididae			Tubificinae	GC	BU	Large	NA
Arthropoda	Arachnida	Sarcoptiformes				Oribatida	PR	NA	Small	NA
Arthropoda	Arachnida	Trombidiformes	Arrenuridae	Arrenurus		Arrenurus	PR	SW	Small	Bi-Multivoltine
Arthropoda	Arachnida	Trombidiformes	Hydrachnidae	Hydrachna		Hydrachna	PR	SW	Small	Univoltine
Arthropoda	Arachnida	Trombidiformes	Hydryphantidae	Hydryphantes		Hydryphantes	PR	SW	Small	Univoltine
Arthropoda	Arachnida	Trombidiformes	Limnesiidae	Limnesia		Limnesia	PR	SW	Small	Univoltine
Arthropoda	Arachnida	Trombidiformes	Pionidae	Piona		Piona	PR	SW	Small	Univoltine
Arthropoda	Arachnida	Trombidiformes	Unionicolidae	Koenikea		Koenikea	PR	SW	Small	Univoltine
Arthropoda	Arachnida	Trombidiformes	Unionicolidae	Neumania		Neumania	PR	SW	Small	Univoltine
Arthropoda	Arachnida	Trombidiformes				Trombidiformes	PR	NA	Small	NA
Arthropoda	Collembola		Isotomidae	Isotoma		Isotoma	GC	NA	Small	NA
Arthropoda	Collembola		Sminthuridae	Dicyrtomina		Dicyrtomina	GC	NA	Small	NA
Arthropoda	Collembola		Sminthuridae			Sminthuridae	GC	NA	Small	NA
Arthropoda	Insecta	Coleoptera	Chrysomelidae			Chrysomelidae	SH	CN	Small	NA
Arthropoda	Insecta	Coleoptera	Curculionidae			Curculionidae	SH	CN	Large	Univoltine
Arthropoda	Insecta	Coleoptera	Dytiscidae			Dytiscidae	PR	SW	Small	Semivoltine
Arthropoda	Insecta	Coleoptera	Dytiscidae			Hydroporinae (subfamily)	PR	SW	Small	Semivoltine
Arthropoda	Insecta	Coleoptera	Dytiscidae	Hydroporus		Hydroporus	PR	SW	Medium	Semivoltine
Arthropoda	Insecta	Coleoptera	Dytiscidae	Hygrotus		Hygrotus	PR	SW	Small	Bi-Multivoltine
Arthropoda	Insecta	Coleoptera	Dytiscidae	Laccophilus		Laccophilus	PR	SW	Small	Bi-Multivoltine
Arthropoda	Insecta	Coleoptera	Dytiscidae	Matus		Matus	PR	SW	Small	Univoltine
Arthropoda	Insecta	Coleoptera	Dytiscidae	Cybister		Cybister	PR	SW	Large	Univoltine
Arthropoda	Insecta	Coleoptera	Dytiscidae	Dytiscus		Dytiscus	PR	SW	Large	Univoltine

Arthropoda	Insecta	Coleoptera	Haliplidae	Haliplus		Haliplus	PI	SW	Small	Bi-Multivoltine
Arthropoda	Insecta	Coleoptera	Haliplidae	Peltodytes		Peltodytes	SH	SW	Small	Univoltine
Arthropoda	Insecta	Coleoptera	Haliplidae	Peltodytes	lengi	Peltodytes lengi	SH	SW	Small	Bi-Multivoltine
Arthropoda	Insecta	Coleoptera	Haliplidae	Peltodytes	sexmaculatus	Peltodytes sexmaculatus	SH	SW	Small	Bi-Multivoltine
Arthropoda	Insecta	Coleoptera	Hydrophilidae	Enochrus		Enochrus	GC	SW	Small	Univoltine
Arthropoda	Insecta	Coleoptera	Hydrophilidae	Hydrochara		Hydrochara	GC	SW	Large	Univoltine
Arthropoda	Insecta	Coleoptera	Hydrophilidae	Tropisternus		Tropisternus	PR	SW	Medium	Bi-Multivoltine
Arthropoda	Insecta	Coleoptera	Hydrophilidae	Tropisternus	blatchleyi	Tropisternus blatchleyi	GC	SW	Medium	Bi-Multivoltine
Arthropoda	Insecta	Coleoptera	Hydrophilidae	Tropisternus	mixtus	Tropisternus mixtus	GC	SW	Medium	Bi-Multivoltine
Arthropoda	Insecta	Coleoptera	Hydrophilidae	Berosus		Berosus	SH	SW	Small	Bi-Multivoltine
Arthropoda	Insecta	Coleoptera	Hydrophilidae	Hydrophilus		Hydrophilus	PR	SW	Large	Univoltine
Arthropoda	Insecta	Coleoptera	Hydrophilidae	Laccobius		Laccobius	PI	SW	Small	Univoltine
Arthropoda	Insecta	Coleoptera	Lampyridae			Lampyridae	PR	BU	Large	NA
Arthropoda	Insecta	Coleoptera	Noteridae			Noteridae	PR	BU	Small	Univoltine
Arthropoda	Insecta	Coleoptera	Noteridae	Hydrocanthus		Hydrocanthus	PR	CB	Small	Univoltine
Arthropoda	Insecta	Coleoptera	Noteridae	Hydrocanthus	iricolor	Hydrocanthus iricolor	PR	CB	Small	Univoltine
Arthropoda	Insecta	Coleoptera	Noteridae	Suphisellus		Suphisellus	PR	CB	Small	Univoltine
Arthropoda	Insecta	Coleoptera	Scirtidae	Cyphon		Cyphon	SC	CB	Small	Univoltine
Arthropoda	Insecta	Coleoptera	Scirtidae	Scirtes		Scirtes	SH	CB	Small	Univoltine
Arthropoda	Insecta	Coleoptera	Staphylinidae			Staphylinidae	PR	CN	Large	NA
Arthropoda	Insecta	Diptera	Ceratopogonidae			Ceratopogoninae (subfamily)	PR	SP	Small	Univoltine
Arthropoda	Insecta	Diptera	Ceratopogonidae	Bezzia		Bezzia	PR	BU	Small	Bi-Multivoltine
Arthropoda	Insecta	Diptera	Chironomidae			Chironomini (tribe)	GC	BU	Small	Bi-Multivoltine
Arthropoda	Insecta	Diptera	Chironomidae	Chironomus		Chironomus	GC	BU	Medium	Univoltine
Arthropoda	Insecta	Diptera	Chironomidae	Chironomus	Decorus Gr.	Chironomus Decorus Gr.	GC	BU	Medium	Univoltine
Arthropoda	Insecta	Diptera	Chironomidae	Cladopelma		Cladopelma	GC	BU	Small	Bi-Multivoltine
Arthropoda	Insecta	Diptera	Chironomidae	Cryptochironomus		Cryptochironomus	PR	SP	Small	Bi-Multivoltine
Arthropoda	Insecta	Diptera	Chironomidae	Cryptochironomus	psittacinus	Cryptochironomus psittacinus	PR	SP	Small	Bi-Multivoltine
Arthropoda	Insecta	Diptera	Chironomidae	Dicrotendipes		Dicrotendipes	GC	BU	Small	Bi-Multivoltine

Arthropoda	Insecta	Diptera	Chironomidae	Endochironomus		Endochironomus	SH	CN	Small	Bi-Multivoltine
Arthropoda	Insecta	Diptera	Chironomidae	Glyptotendipes		Glyptotendipes	GC	BU	Small	Univoltine
Arthropoda	Insecta	Diptera	Chironomidae	Hyporhygma	quadripunctatus	Hyporhygma quadripunctatus	SH	BU	Small	Bi-Multivoltine
Arthropoda	Insecta	Diptera	Chironomidae	Kiefferulus		Kiefferulus	GC	BU	Small	Bi-Multivoltine
Arthropoda	Insecta	Diptera	Chironomidae	Parachironomus		Parachironomus	GC	SP	Small	Bi-Multivoltine
Arthropoda	Insecta	Diptera	Chironomidae	Polypedilum	Fallax Gr.	Polypedilum Fallax Gr.	GC	CB	Small	Bi-Multivoltine
Arthropoda	Insecta	Diptera	Chironomidae	Polypedilum	Halterale Gr.	Polypedilum Halterale Gr.	GC	CB	Small	Bi-Multivoltine
Arthropoda	Insecta	Diptera	Chironomidae	Polypedilum	Illinoense Gr.	Polypedilum Illinoense Gr.	GC	CB	Small	Bi-Multivoltine
Arthropoda	Insecta	Diptera	Chironomidae	Pseudochironomus		Pseudochironomus	GC	BU	Small	Bi-Multivoltine
Arthropoda	Insecta	Diptera	Chironomidae	Paratanytarsus		Paratanytarsus	GC	SP	Small	Bi-Multivoltine
Arthropoda	Insecta	Diptera	Chironomidae	Tanytarsus	Glabrescens Gr.	Tanytarsus Glabrescens Gr.	GC	CB	Small	Bi-Multivoltine
Arthropoda	Insecta	Diptera	Chironomidae	Zavreliella	marmorata	Zavreliella marmorata	GC	BU	Small	Bi-Multivoltine
Arthropoda	Insecta	Diptera	Chironomidae			Chironominae	GC	BU	Small	Bi-Multivoltine
Arthropoda	Insecta	Diptera	Chironomidae	Cricotopus		Cricotopus (Isocladius)	SH	CN	Small	Bi-Multivoltine
Arthropoda	Insecta	Diptera	Chironomidae	Cricotopus	Reversus Gr.	Cricotopus Reversus Gr.	SH	CN	Small	Bi-Multivoltine
Arthropoda	Insecta	Diptera	Chironomidae	Cricotopus	Sylvestris Gr.	Cricotopus Sylvestris Gr.	SH	CN	Small	Bi-Multivoltine
Arthropoda	Insecta	Diptera	Chironomidae	Nanocladius	crassicornus/rectinervis	Nanocladius crassicornus/rectinervis complex	GC	SP	Small	Univoltine
Arthropoda	Insecta	Diptera	Chironomidae	Parakiefferiella	coronata	Parakiefferiella coronata	GC	SP	Small	Univoltine
Arthropoda	Insecta	Diptera	Chironomidae			Orthocladiinae	GC	BU	Small	Univoltine
Arthropoda	Insecta	Diptera	Chironomidae	Clinotanypus		Clinotanypus	PR	BU	Small	Univoltine
Arthropoda	Insecta	Diptera	Chironomidae	Ablabesmyia		Ablabesmyia	PR	SP	Small	Univoltine
Arthropoda	Insecta	Diptera	Chironomidae	Ablabesmyia	peleensis	Ablabesmyia peleensis	PR	SP	Small	Univoltine
Arthropoda	Insecta	Diptera	Chironomidae	Ablabesmyia (Karelia)		Ablabesmyia (Karelia)	PR	SP	Small	Univoltine
Arthropoda	Insecta	Diptera	Chironomidae	Guttipelopia	guttipennis	Guttipelopia guttipennis	PR	SP	Small	Univoltine

Arthropoda	Insecta	Diptera	Chironomidae	Labrundinia		Labrundinia	PR	SP	Small	Univoltine
Arthropoda	Insecta	Diptera	Chironomidae	Labrundinia	neopilosella	Labrundinia neopilosella	PR	SP	Small	Univoltine
Arthropoda	Insecta	Diptera	Chironomidae	Larsia		Larsia	PR	SP	Small	Univoltine
Arthropoda	Insecta	Diptera	Chironomidae	Zavrelimyia		Zavrelimyia	PR	SP	Small	Univoltine
Arthropoda	Insecta	Diptera	Chironomidae	Procladius (Holotanypus)		Procladius (Holotanypus)	PR	SP	Small	Univoltine
Arthropoda	Insecta	Diptera	Chironomidae	Tanypus		Tanypus	PR	SP	Small	Univoltine
Arthropoda	Insecta	Diptera	Chironomidae	Tanypus	carinatus	Tanypus carinatus	GC	SP	Small	Univoltine
Arthropoda	Insecta	Diptera	Chironomidae	Tanypus	eopunctipenni	Tanypus neopunctipennis	PR	SP	Small	Univoltine
Arthropoda	Insecta	Diptera	Chironomidae	Tanypus	unctipennis	Tanypus punctipennis	PR	SP	Small	Univoltine
	Insecta	Diptera	Chironomidae				PR	SP	Small	Univoltine
Arthropoda	Insecta	Diptera	Chironomidae			Chironomidae	GC	SP	Small	Univoltine
Arthropoda	Insecta	Diptera	Culicidae			Culicidae	FC	SW	Medium	Univoltine
Arthropoda	Insecta	Diptera	Culicidae	Anopheles		Anopheles	FC	SW	Small	Bi-Multivoltine
Arthropoda	Insecta	Diptera	Culicidae	Culex		Culex	FC	SW	Medium	Bi-Multivoltine
Arthropoda	Insecta	Diptera	Culicidae	Uranotaenia		Uranotaenia	FC	SW	Small	Univoltine
Arthropoda	Insecta	Diptera	Culicidae	Culiseta		Culiseta	GC	SW	Medium	Univoltine
Arthropoda	Insecta	Diptera	Culicidae	Mansonia		Mansonia	GC	CN	Medium	Univoltine
Arthropoda	Insecta	Diptera	Dixidae	Dixella		Dixella	GC	SW	Small	Univoltine
Arthropoda	Insecta	Diptera	Ephydridae			Ephydridae	GC	BU	Medium	Bi-Multivoltine
Arthropoda	Insecta	Diptera	Psychodidae	Pericoma		Pericoma	GC	BU	Small	Univoltine
Arthropoda	Insecta	Diptera	Sciomyzidae			Sciomyzidae	PR	BU	Medium	Bi-Multivoltine
Arthropoda	Insecta	Diptera	Stratiomyidae			Stratiomyini (tribe)	GC	SP	Medium	NA
Arthropoda	Insecta	Diptera	Stratiomyidae	Stratiomys		Stratiomys	GC	SP	Medium	NA
Arthropoda	Insecta	Diptera	Tipulidae	Angarotipula		Angarotipula	SH	BU	Medium	Univoltine
Arthropoda	Insecta	Diptera	Tipulidae	Tipula		Tipula	SH	BU	Medium	Univoltine
Arthropoda	Insecta	Diptera	Tipulidae			Tipulidae	SH	BU	Medium	Univoltine
Arthropoda	Insecta	Hemiptera	Belostomatidae	Belostoma	flumineum	Belostoma flumineum	PR	SW	Large	Bi-Multivoltine
Arthropoda	Insecta	Hemiptera	Corixidae			Corixidae	PI	SW	Small	Bi-Multivoltine
Arthropoda	Insecta	Hemiptera	Corixidae	Hesperocorixa	laevigata	Hesperocorixa laevigata	PI	SW	Medium	Univoltine
Arthropoda	Insecta	Hemiptera	Corixidae	Hesperocorixa	lucida	Hesperocorixa lucida	PI	SW	Medium	Univoltine
Arthropoda	Insecta	Hemiptera	Corixidae	Hesperocorixa	semilucida	Hesperocorixa semilucida	PI	SW	Medium	Univoltine
Arthropoda	Insecta	Hemiptera	Corixidae	Palmacorixa		Palmacorixa	PI	SW	Small	Bi-Multivoltine

Arthropoda	Insecta	Hemiptera	Corixidae	Palmacorixa	buenoi	Palmacorixa buenoi	PI	SW	Small	Bi-Multivoltine
Arthropoda	Insecta	Hemiptera	Corixidae	Sigara	alternata	Sigara alternata	SC	SW	Small	Bi-Multivoltine
Arthropoda	Insecta	Hemiptera	Corixidae	Trichocorixa		Trichocorixa	PR	SW	Small	Bi-Multivoltine
Arthropoda	Insecta	Hemiptera	Corixidae	Trichocorixa	borealis	Trichocorixa borealis	PR	SW	Small	Bi-Multivoltine
Arthropoda	Insecta	Hemiptera	Corixidae	Trichocorixa	sexcincta	Trichocorixa sexcincta	PR	SW	Small	Bi-Multivoltine
Arthropoda	Insecta	Hemiptera	Corixidae	Hesperocorixa		Hesperocorixa	PI	SW	Medium	Univoltine
Arthropoda	Insecta	Hemiptera	Gerridae			Gerridae	PR	SK	Medium	NA
Arthropoda	Insecta	Hemiptera	Gerridae	Rheumatobates		Rheumatobates	PR	SK	Small	Univoltine
Arthropoda	Insecta	Hemiptera	Hebridae	Merragata		Merragata	PR	SK	Small	Bi-Multivoltine
Arthropoda	Insecta	Hemiptera	Hebridae	Lipogomphus		Lipogomphus	PR	CB	Small	NA
	Insecta	Hemiptera	Hydrometridae	Hydrometra		Hydrometra	PI	SK	Medium	Bi-Multivoltine
Arthropoda	Insecta	Hemiptera	Macroveliidae	Macrovelia		Macrovelia	PI	CB	Small	NA
Arthropoda	Insecta	Hemiptera	Mesoveliidae	Mesovelia		Mesovelia	PI	SK	Small	Bi-Multivoltine
Arthropoda	Insecta	Hemiptera	Naucoridae	Pelocoris	femoratus	Pelocoris femoratus	PI	CB	Medium	NA
Arthropoda	Insecta	Hemiptera	Nepidae	Ranatra	nigra	Ranatra nigra	PI	CN	Large	NA
Arthropoda	Insecta	Hemiptera	Nepidae	Ranatra		Ranatra	PI	CN	Large	NA
Arthropoda	Insecta	Hemiptera	Notonectidae			Notonectidae	PI	SW	Medium	NA
Arthropoda	Insecta	Hemiptera	Notonectidae	Buenoa		Buenoa	PI	SW	Small	NA
Arthropoda	Insecta	Hemiptera	Notonectidae	Buenoa	confusa	Buenoa confusa	PI	SW	Small	NA
Arthropoda	Insecta	Hemiptera	Notonectidae	Notonecta		Notonecta	PI	SW	Large	Univoltine
Arthropoda	Insecta	Hemiptera	Pleidae	Neoplea	striola	Neoplea striola	PI	SW	Small	NA
Arthropoda	Insecta	Hemiptera	Veliidae	Microvelia		Microvelia	PI	SK	Small	Bi-Multivoltine
Arthropoda	Insecta	Hemiptera	Veliidae	Platyvelia		Platyvelia	PI	SK	Small	NA
Arthropoda	Insecta	Odonata	Aeshnidae			Aeshnidae	PR	CN	Large	Univoltine
Arthropoda	Insecta	Odonata	Aeshnidae	Anax	junius	Anax junius	PR	CN	Large	Univoltine
Arthropoda	Insecta	Odonata	Coenagrionidae	Enallagma		Enallagma	PR	CB	Medium	Univoltine
Arthropoda	Insecta	Odonata	Coenagrionidae	Ischnura		Ischnura	PR	CB	Medium	Univoltine
Arthropoda	Insecta	Odonata	Coenagrionidae			Coenagrionidae	PR	CN	Medium	Univoltine
Arthropoda	Insecta	Odonata	Corduliidae	Helocordulia		Helocordulia	PR	SP	Medium	Semivoltine
Arthropoda	Insecta	Odonata	Lestidae	Lestes	inaequalis	Lestes inaequalis	PR	CB	Large	Univoltine
Arthropoda	Insecta	Odonata	Libellulidae			Libellulidae	PR	SP	Large	Univoltine
Arthropoda	Insecta	Odonata	Libellulidae	Celithemis	eponina	Celithemis eponina	PR	CB	Large	Univoltine
Arthropoda	Insecta	Odonata	Libellulidae	Erythemis	simplicicollis	Erythemis simplicicollis	PR	CB	Large	Univoltine
Arthropoda	Insecta	Odonata	Libellulidae	Pachydiplax	longipennis	Pachydiplax longipennis	PR	SP	Large	Univoltine

Arthropoda	Insecta	Odonata	Libellulidae	Sympetrum		Sympetrum	PR	SP	Large	Univoltine
Arthropoda	Insecta	Odonata	Libellulidae	Sympetrum	vicinum	Sympetrum vicinum	PR	SP	Large	Univoltine
Arthropoda	Insecta	Odonata	Libellulidae	Tramea	lacerata	Tramea lacerata	PR	SP	Large	Univoltine
Arthropoda	Insecta	Odonata	Libellulidae	Erythemis		Erythemis	PR	CB	Large	Univoltine
Arthropoda	Insecta	Odonata	Libellulidae	Libellula		Libellula	PR	SP	Large	Univoltine
Arthropoda	Insecta	Odonata				Anisoptera	PR	CB	Large	NA
Arthropoda	Insecta	Trichoptera	Brachycentridae	Brachycentrus		Brachycentrus	FC	CN	Medium	Univoltine
Arthropoda	Insecta	Trichoptera	Hydroptilidae	Agraylea		Agraylea	SH	CB	Small	Univoltine
Arthropoda	Insecta	Trichoptera	Hydroptilidae	Oxyethira		Oxyethira	SH	CB	Small	Univoltine
Arthropoda	Insecta	Trichoptera	Hydroptilidae	Orthotrichia		Orthotrichia	SC	CN	Small	Univoltine
Arthropoda	Insecta	Trichoptera	Hydroptilidae			Hydroptilidae	PI	CN	Small	Univoltine
Arthropoda	Insecta	Trichoptera	Leptoceridae	Leptocerus	americanus	Leptocerus americanus	SH	SW	Medium	Univoltine
Arthropoda	Insecta	Trichoptera	Leptoceridae	Triaenodes		Triaenodes	SH	SW	Medium	Univoltine
Arthropoda	Insecta	Trichoptera	Leptoceridae	Ceraclea		Ceraclea	GC	SP	Medium	Univoltine
Arthropoda	Insecta	Trichoptera	Leptoceridae	Oecetis		Oecetis	PR	CN	Medium	Univoltine
Arthropoda	Insecta	Trichoptera	Phryganeidae	Fabria	inornata	Fabria inornata	SH	CB	Large	NA
Arthropoda	Insecta	Trichoptera	Polycentropodidae			Polycentropodidae	FC	CN	Medium	NA
Arthropoda	Insecta	Trichoptera	Polycentropodidae	Neureclipsis		Neureclipsis	FC	CN	Medium	Bi-Multivoltine
Arthropoda	Insecta	Trichoptera	Polycentropodidae	Polycentropus		Polycentropus	FC	CN	Medium	Univoltine
Arthropoda	Insecta	Trichoptera				Trichoptera	GC	NA	Large	NA
Arthropoda	Insecta	Ephemeroptera	Baetidae	Callibaetes		Callibaetis	GC	SW	Small	Bi-Multivoltine
Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	Diminuta Gr.	Caenis Diminuta Gr.	GC	SP	Small	Univoltine
Arthropoda	Insecta	Lepidoptera	Crambidae			Crambidae	SH	CB	Large	NA
Arthropoda	Insecta	Lepidoptera	Noctuidae			Noctuidae	SH	BU	Large	NA
Arthropoda	Insecta	Megaloptera	Corydalidae	Chauliodes		Chauliodes	PR	CN	Large	Semivoltine
Arthropoda	Insecta	Megaloptera	Corydalidae	Nigronia		Nigronia	PR	CN	Large	Semivoltine
Arthropoda	Insecta	Megaloptera	Sialidae	Sialis		Sialis	PR	BU	Medium	Univoltine
Arthropoda	Malacostraca	Amphipoda	Crangonyctidae	Crangonyx		Crangonyx	GC	SP	Medium	Univoltine
Arthropoda	Malacostraca	Amphipoda	Gammaridae			Gammarus	GC	SP	Large	Univoltine
Arthropoda	Malacostraca	Amphipoda	Gammaridae	Echinogammarus	ischnus	Echinogammarus ischnus	PR	NA	Large	Bi-Multivoltine
Arthropoda	Malacostraca	Amphipoda	Hyalellidae	Hyalella		Hyalella	GC	SP	Small	NA
Arthropoda	Malacostraca	Decapoda	Cambaridae	Faxonius		Faxonius	GC	CB	Large	NA
Arthropoda	Malacostraca	Decapoda	Cambaridae	Fallicambarus		Fallicambarus	GC	BU	Large	NA
Arthropoda	Malacostraca	Decapoda	Cambaridae	Orconectes		Orconectes	GC	CB	Large	NA

Arthropoda	Malacostraca	Decapoda	Cambaridae	Procambarus		Procambarus	GC	CB	Large	NA
Arthropoda	Malacostraca	Decapoda	Palaemonidae	Palaemon		Palaemon	GC	NA	Large	NA
Arthropoda	Malacostroca	Isopoda	Asellidae	Caecidotea		Caecidotea	GC	CN	Medium	NA
Mollusca	Bivalvia	Veneroida	Dreissenidae	Dreissena	polymorpha	Dreissena polymorpha	FC	CB	Large	Univoltine
Mollusca	Bivalvia	Veneroida	Sphaeriidae	Pisidium		Pisidium	FC	BU	Small	Bi-Multivoltine
Mollusca	Bivalvia	Veneroida	Sphaeriidae	Sphaerium		Sphaerium	FC	BU	Large	Bi-Multivoltine
Mollusca	Gastropoda	Basommatophora	Lymnaeidae	Pseudosuccinea	columella	Pseudosuccinea columella	GC	CB	Large	NA
Mollusca	Gastropoda	Basommatophora	Lymnaeidae	Stagnicola		Stagnicola	GC	CB	Large	Univoltine
Mollusca	Gastropoda	Basommatophora	Lymnaeidae	Stagnicola	elodes	Stagnicola elodes	GC	CB	Large	Univoltine
Mollusca	Gastropoda	Basommatophora	Lymnaeidae	Galba		Galba	SC	CB	Medium	NA
Mollusca	Gastropoda	Basommatophora	Lymnaeidae	Lymnaea		Lymnaea	SC	CB	Large	NA
Mollusca	Gastropoda	Basommatophora	Lymnaeidae	Radix		Radix	GC	CB	Large	Univoltine
Mollusca	Gastropoda	Basommatophora	Physidae	Physella		Physella	SC	CB	Large	Univoltine
Mollusca	Gastropoda	Basommatophora	Physidae	Physella	acuta	Physella acuta	SC	CB	Large	Univoltine
Mollusca	Gastropoda	Basommatophora	Physidae	Physella	gyrina	Physella gyrina	SC	CB	Large	Univoltine
Mollusca	Gastropoda	Basommatophora	Physidae	Aplexa		Aplexa	SC	NA	Medium	NA
Mollusca	Gastropoda	Basommatophora	Planorbidae			Planorbidae	SC	CB	Large	NA
Mollusca	Gastropoda	Basommatophora	Planorbidae	Ferrissia		Ferrissia	SC	CB	Small	Univoltine
Mollusca	Gastropoda	Basommatophora	Planorbidae	Gyraulus		Gyraulus	SC	CB	Medium	NA
Mollusca	Gastropoda	Basommatophora	Planorbidae	Micromenetus	dilatatus	Micromenetus dilatatus	SC	CB	Small	NA
Mollusca	Gastropoda	Basommatophora	Planorbidae	Planorbella		Planorbella	SC	CB	Large	NA
Mollusca	Gastropoda	Basommatophora	Planorbidae	Planorbella	trivolvis	Planorbella trivolvis	SC	CB	Large	NA
Mollusca	Gastropoda	Basommatophora	Planorbidae	Helisoma		Helisoma	SC	CB	Large	NA
Mollusca	Gastropoda	Basommatophora	Planorbidae	Menetus		Menetus	SC	CB	Small	Univoltine
Mollusca	Gastropoda	Neotaenioglossa	Pleuroceridae	Lithasia		Lithasia	SC	NA	Medium	NA
Mollusca	Gastropoda	Stylommatophora	Succineidae	Succinea		Succinea	SC	NA	Large	NA
Mollusca	Gastropoda	Heterostropha	Valvatidae	Valvata		Valvata	SC	SP	Medium	Univoltine
Molluska	Gastropoda	Architaenioglossa	Viviparidae	Cipangopaludina		Cipangopaludina	SC	NA	Large	NA
Platyhelminthes						Trepaxonemata (subclass)	PR	NA	Large	NA

Appendix B. Chapter 1 summary

Table B. 1 Mean and standard error of water quality parameters and nutrients collected in restored and unrestored wetlands in 2016 and 2017.

	2016		**2017**	
	Restored	**Unrestored**	**Restored**	**Unrestored**
TN (mg/L)	0.58 (±0.06)	1.36 (±0.13)	0.99 (±0.15)	1.20 (±0.10)
TP (mg/L)	0.11 (±0.01)	0.12 (±0.01)	0.04 (±0.01)	0.05 (±0.005)
Nitrate-N (mg/L)	0.07 (±0.01)	0.29 (±0.06)	0.14 (±0.06)	0.14 (± 0.08)
Ammonia-N (mg/L)	0.03 (±0.01)	0.01 (±0.003)	0.28 (±0.21)	0.01 (±0.01)
Phosphate-P (mg/L)	0.04 (±0.01)	0.08 (±0.02)	0.04 (±0.02)	0.03 (±0.01)
TN:TP	6.75 (±1.02)	12.57 (±1.19)	24.85 (±2.52)	29.44 (±1.98)
Temperature (°C)	24.26 (±0.37)	26.25 (±0.45)	21.60 (±0.63)	21.48 (±0.41)
Conductivity (µS/cm)	70.82 (±12.68)	49.80 (±7.32)	0.41 (±0.03)	0.31 (±0.01)
Turbidity (NTUs)	7.58 (±0.97)	23.97 (±2.24)	11.70 (±1.83)	17.25 (±1.90)
DO (%)	11.70 (±3.0)	17.30 (±2.87)	84.67 (±6.42)	85.01 (±6.92)
pH	7.59 (±0.09)	7.90 (±0.13)	8.01 (±0.08)	8.09 (±0.10)

Table B. 2 Mean and standard error of macroinvertebrate metrics in restored and unrestored wetlands in 2016 and 2017.

	2016		**2017**	
	Restored	**Unrestored**	**Restored**	**Unrestored**
Taxonomic richness	35.17 (±2.38)	34.96 (±1.97)	34.67 (±1.97)	35.52 (±1.02)
Shannon diversity	2.67 (±0.06)	2.62 (±0.07)	2.58 (±0.06)	2.62 (±0.05)
EOT richness	6.58 (±0.42)	6.33 (±0.33)	6.53 (±0.65)	6.00 (±0.38)
Percent contribution dominant taxa	22.36 (±1.41)	25.48 (±1.57)	24.10 (±2.08)	23.55 (±1.81)
FC	0.45 (±0.19)	0.18 (±0.06)	0.21 (±0.06)	0.86 (±0.13)
GC	52.87 (±2.23)	49.56 (±3.12)	48.80 (±2.69)	51.99 (±2.09)
PI	5.13 (±0.77)	13.35 (±2.27)	6.52 (±1.19)	11.59 (±1.79)
PR	27.00 (±2.42)	29.18 (±1.87)	30.95 (±2.51)	25.25 (±1.82)
SC	13.60 (±2.48)	7.24 (±1.00)	12.84 (±2.67)	9.27 (±1.89)
SH	0.96 (±0.34)	0.48 (±0.12)	0.70 (±0.16)	1.04 (±0.38)
BU	12.63 (±2.65)	13.61 (±1.79)	18.42 (±4.07)	13.54 (±2.73)
CB	17.41 (±2.11)	13.94 (±1.35)	19.10 (±2.39)	15.53 (±1.89)
CN	8.71 (±1.85)	8.07 (±1.45)	6.93 (±1.88)	6.79 (±1.61)
SK	3.95 (±0.80)	3.17 (±0.50)	2.75 (±0.76)	2.37 (±0.54)
SP	51.53 (±2.45)	42.41 (±3.07)	44.08 (±4.06)	45.51 (±2.44)
SW	5.76 (±1.13)	18.80 (±3.15)	8.72 (±2.38)	16.26 (±2.36)
Small taxa	52.88 (±2.74)	62.33 (±2.10)	52.14 (±3.54)	61.26 (±2.14)
Medium taxa	29.34 (±2.27)	22.26 (±2.13)	29.72 (±2.52)	23.32 (±1.45)
Large taxa	17.78 (±2.33)	15.42 (±1.37)	18.14 (±2.21)	15.42 (±1.68)
Semivoltine taxa	0.37 (±0.14)	0.16 (±0.10)	0.02 (±0.02)	0.14 (±0.07)
Univoltine taxa	76.61 (±3.02)	68.88 (±2.99)	68.66 (±3.55)	68.58 (±2.37)
Bi-multivoltine taxa	23.02 (±3.06)	30.96 (±3.00)	31.31 (±3.56)	31.27 (±2.36)
Functional richness	0.0001 (±0.00005)	0.0001 (±0.00003)	0.0002 (±0.0002)	0.0001 (±0.00002)
RQE	0.24 (±0.01)	0.23 (±0.01)	0.24 (±0.01)	0.24 (±0.01)

Appendix C. Chapter 2 summary

Table C. 1 Generalized habitat requirements of amphibian species recorded from Great Lakes Marsh Monitoring Program in Ottawa County. Monitoring in Ottawa County began in 1996 and data have not been updated since 2014. Modified from Hecnar (2004). A = aquatic, T = terrestrial.

		Life-cycle stage				**Activity of adult**				
Common Name	**First and last year recorded**	**Egg**	**Larvae**	**Juvenile**	**Adult**	**Hibernation**	**Breeding**	**Foraging**	**Migration**	**Affinities**
Northern leopard frog	1996, 2014	A	A	T, A	T, A	A	A	T	T, A	Grassland, deep water
Chorus frogs	1996, 2014	A	A	T	T	T	A	T	T	Forest
Wood frog	1999, 2014	A	A	T	T	T	A	T	T	Moist forest
American toad	1998, 2014	A	A	T	T	T	A	T	T	Habitat generalists
American bullfrog	1996, 2014	A	A	A	A	A	A	A	A, T	Deep permanent water
Green frog	1996, 2014	A	A	A, T	A, T	A	A	T, A	T, A	Deep permanent water
Spring peeper	1996, 2014	A	A	T	T	T	A	T	T	Forest, shrub, marshes
Gray treefrog	2000, 2014	A	A	T	T	T	A	T	T	Forest, shrubs
Pickerel frog	1996, 2014	A	A	A, T	A	A	A	A, T	T, A	Deep cold water
Fowler's toad	2002, 2013	A	A	T	T	T	A	T	T	Open habitat, lakeshores, sand
Blanchard's cricket frog	1999, 2011	A	A	T, A	T, A	T	A	T, A	T, A	Open edges, permanent waters

Table C. 2 Beaufort wind scale.

0	Calm; smoke rises vertically
1	Light air movement; smoke drifts; leaves barely move
2	Slight breeze; wind felt on face; small twigs move
3	Gentle breeze; leaves & small twigs in constant motion
4	Moderate breeze; small branches moving, raises dust & loose paper
5	Large branches & small trees sway

Table C. 3 Noise codes.

0	No appreciable effect (owl calling)
1	Slightly affecting sampling (distant traffic, dog barking, car passing)
2	Moderately affecting sampling (distant traffic, 2-5 cars passing)
3	Seriously affecting sampling (continuous traffic nearby, 6-10 cars passing)
4	Profoundly affecting sampling (continuous traffic passing, construction noise)

Table C. 4 Weather.

1	Dry
2	Damp/haze/fog
3	Drizzle/rain

Table C. 5 Calling codes.

1	Calls not simultaneous; individuals can be accurately counted
2	Some calls simultaneous; individuals can be reliably estimated
3	Full chorus, calls continuous & overlapping; not reliably estimated

Table C. 6 Mean and standard error of water quality parameters and wetland characteristics in restored and unrestored wetlands.

	Restored	**Unrestored**
Temperature (°C)	21.6 (±0.63)	21.48 (±0.41)
Conductivity (µS/cm)	0.41 (±0.03)	0.31 (±0.01)
Turbidity (NTUs)	11.7 (±1.83)	17.25 (±1.90)
DO (%)	84.67 (±6.42)	85.01 (±6.92)
pH	8.01 (±0.08)	8.09 (±0.10)
Nitrate-N (mg/L)	0.14 (±0.06)	0.14 (±0.08)
TP (mg/L)	0.04 (±0.01)	0.05 (±0.004)
AFDM (mg/cm^2)	8.51E-06 (±2.13E-06)	1.63E-05 (±1.77E-06)
Plant richness	9.4 (±1.50)	13.14 (±0.94)
Plant biomass (g/m^2)	1602 (±214)	1843 (±270)

Table C. 7 Summary of amphibians trapped in restored and unrestored wetlands.

	Restored			Unrestored		
	Tadpole	Adult	Total	Tadpole	Adult	Total
American bullfrog	34	7	41	90	11	101
Northern leopard frog	17	0	17	3	0	3
Green frog	3	1	4	16	1	17
American toad	0	0	0	117	0	117
Total	54	8	62	226	12	238

Table C. 8 Mean and standard error of tadpole CPUE in restored and unrestored wetlands.

	Restored	Unrestored
American bullfrog tadpole CPUE	0.012 (±0.01)	0.019 (±0.01)
Northern leopard frog tadpole CPUE	0.005 (±0.004)	0.001 (±0.0004)
Green frog tadpole CPUE	0.001 (±0.001)	0.003 (±0.002)
Total tadpole CPUE	0.015 (±0.01)	0.022 (±0.01)

Table C. 9 Mean and standard error of amphibian call codes in restored (R) and unrestored (UR) wetlands across sampling trips.

Status	R	UR	R	UR	R	UR
Sampling Trip	1	1	2	2	3	3
American bullfrog	2.8 (±1.24)	5 (±0.85)	1.6 (±0.5)	1.24 (±0.24)	0	0.29 (±0.12)
Green frog	1.1 (±0.49)	3.1 (±1.17)	1.55 (±0.40)	2.41 (±0.53)	0.88 (±0.23)	1.11 (±0.24)
American toad	0	0	0	0.29 (±0.2)	1.2 (±0.31)	0.43 (±0.11)
Gray treefrog	0	0	0	0	1 (±0.3)	0.52 (±0.2)

Table C. 10 Mean and standard error of tadpole biomass (g/wetland) in restored and unrestored wetlands.

	Restored	**Unrestored**
American bullfrog tadpole biomass	7.1 (±3.98)	31.02 (±13.34)
Northern leopard frog tadpole biomass	4.47 (±3.85)	0.5 (±0.29)
Green frog tadpole biomass	0.7 (±0.7)	3.31 (±2.85)