Biological Survey of a Newly Constructed Wetland Complex at Oakwoods Nature Preserve (Hancock County, Ohio)

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Abstract: In 2021, 43 wetland pools were created at Oakwoods Nature Preserve with funding from the H2Ohio program. One year post-construction, we conducted biodiversity assessments for benthic bacteria, periphyton, macroalgae, macroinvertebrates, amphibians, reptiles, and fish in all 43 pools. To date, we have identified over 23,000 unique bacterial taxa, 81 algal genera, 51 macroinvertebrate families, and hylid and ranid frogs, as well as centrarchid and cyprinid fishes. This survey indicates an impressive rate of biological colonization one year after pool construction. Long-term biodiversity monitoring of these wetlands will provide important data to inform best management practices for wetland construction and maintenance.

Keywords: benthic bacteria, periphyton, macroinvertebrates, amphibians, fish, restoration ecology

INTRODUCTION

Wetlands are a critically important aspect of the environment, as they host a vast amount of biodiversity. Wetlands are home to a third of all vertebrate species on the planet, and despite only making up less than 1% of the total land area, wetland habitats host 10% of all species on Earth (Dudgeon et al. 2006, Balian et al. 2008). Wetlands also provide critical functions for both the environment and society. Wetlands purify water, recharge aquifers, and provide a nutrient sink in floodplains, preventing nutrient runoff into aquatic systems (WWF 2018). Wetlands also provide recreational value for a variety of activities, such as hiking and fishing. However, wetlands are being degraded at an alarming rate. Some consequences of degraded wetlands are decreased watershed quality, eutrophication, algal blooms, and associated greenhouse gas emissions (Wynne et al. 2021, Liu et al. 2021).

Ohio wetlands in particular are at an increased risk of degradation. Over 90% of Ohio's wetlands have been destroyed due to agriculture and land development (Ohio EPA Division of Surface Water 2016). Since the mid-1990s, Lake Erie has experienced increased phosphorus loading, leading to harmful algal blooms and hypoxic conditions (Scavia et al. 2014). Hypoxic conditions can lead to the extirpation of cold-water fishes and a variety of macroinvertebrates (Scavia et al. 2014). The state of Ohio has introduced measures, including the H2Ohio plan, to combat eutrophication and improve water quality within the state. H2Ohio aims to decrease harmful algal blooms, improve wastewater infrastructure, and decrease lead contamination. The Ohio General Assembly awarded H2Ohio $172,000,000 in 2019 and by fiscal year 2024–2025 has increased this support to $270,000,000 for various H2Ohio projects, including wetland and associated habitat restoration (Ohio Division of Natural Resources H2Ohio 2025).

A large-scale wetland complex funded by H2Ohio was constructed at Oakwoods Nature Preserve in Hancock County in 2020 on former agricultural land. Ground broke for the project on January 11, 2021, and was completed by December 2021. Beyond land excavation and resurfacing, extensive revegetation was conducted with native species by means of hydroseeding, live stakes, and plugs. This wetland complex restored the floodplain of Aurand Run and reconnected this tributary to the Blanchard River. The total area of the project encompasses 142 acres, with 43 newly constructed wetland pools and an existing pool adjacent to a woodlot (Fig. 1). The project is divided into two sections: East (65 acres with 23 pools) and West (77 acres with 19 pools). The primary objective of this project was to conduct

Fig. 1. Overhead view of wetland pools at Oakwoods Nature Preserve, Hancock County, Ohio.

a broad biological survey of the wetland complex one year post-construction. This survey included benthic bacteria, periphyton, macroalgae, macroinvertebrates, amphibians, reptiles, and fish.

METHODS

Field collections were conducted in June 2022. Specific field and laboratory protocols for selected taxonomic groups can be found below. Preserved samples and voucher specimens were deposited in the Ohio Northern University Teaching Museum and Herbarium.

Benthic Bacteria. Benthic soil samples were collected from each pool using 1000µl sterile pipette tips (three replicate samples for each pool) and frozen in the field on dry ice until transferred to a deep freezer (-80ºC) for storage and subsequent laboratory processing (Carino-Kyker and Swanson 2008). Triplicate samples for each pool were homogenized and a DNeasy PowerSoil Pro Kit (Qiagen™) was used to extract total genomic DNA from core samples. Polymerase Chain Reaction (PCR) was used to amplify the 16s rRNA gene region of bacterial DNA in the samples (Carino-Kyker and Swanson 2008). The V4 of the 16S rRNA bacterial gene region was sequenced utilizing Illumina MiSeq platform (University of Michigan's Center for Microbial Systems). DNA was amplified in each sample using the dual-indexing sequencing strategy (Kozich et al.2013). Mothur (v.1.48.0) was used to process sequences and provide a taxa list for this survey (Schloss et al. 2009).

Periphyton. Epipelic periphyton was collected from the uppermost sediments of the pools. Depth below the water surface was standardized at 15 cm. At each pool, sediments were collected using a 5.0 cm^2 o-ring and disposable pipette. For each pool, all the sediments were combined into a composite sample and rinsed into a 50 mL Falcon tube, and preserved with 80% ethanol. For each periphyton sample, a small, homogenized quantity was placed in a Palmer-Maloney counting chamber to 1) enumerate and identify 300 soft-bodied algal cells and 2) survey the abundance and condition of diatoms. Non-diatom (soft-bodied) algae were identified to genus when possible using taxonomic references including Dillard (1999), Prescott (1962), and Whitford and Schumacher (1984), with updated taxonomy from Wehr et al. (2015). To identify diatoms to genus, a subsample (10 ml) from each collection was boiled with 35% hydrogen peroxide for 60 minutes. A series of distilled water dilutions removed oxidation byproducts, after which samples were evaporated onto coverslips and mounted on microscope slides using the mounting medium Zrax™. A minimum of 300 diatom valves were identified to genus and enumerated along a transect(s) using a Meiji MX4300L brightfield light microscope (n.a.=1.30). Identification of diatom genera was based on taxonomic literature including work from the United States (Krammer and Lange-Bertalot 1986, 1988, 1991a, 1991b; Patrick and Reimer 1966, 1975; Spaulding et al. 2021).

Macroalgae. Macroalgae were defined as benthic or part of the metaphyton with a discrete structure recognizable to the naked eye (Dodd 1991, Stock and Ward 1991, Sheath and Cole 1992). Representative samples of each macroalgal taxon were collected from the Oakwoods pools using forceps and placed in a Whirl-Pak™ in which the specimens were preserved with 80% ethanol. In the laboratory, macroalgae were examined using a Meiji MX4300L brightfield light microscope and identified to the genus level using Prescott (1962), Taft and Taft (1971), Whitford and Schumacher (1984), and Wehr et al. (2015).

Macroinvertebrates. Timed dip netting was performed with standardized constructed 500 µm mesh dip nets, with time being standardized by the surface area of the ponds (Dananay et al. 2015). Once sampling was completed, the samples were rinsed in the field with a 500 µm brass sieve and macroinvertebrates were placed in a Whirl-Pak™ and stored in 75% ethanol. In the laboratory, macroinvertebrates were examined using Meiji EMZ-13 stereoscopes. Insects were identified at the family level; other invertebrates were identified to class or order using Voshell (2002), Merritt et al. (2019), and Thorp and Rodgers (2015).

Vertebrates. Amphibian larvae and fish fry were denoted as present or absent at each pool during the timed dip netting (described above). Electroshocking for adult fish was completed in the nine pools (4, 9, 18, 34, 42, 29, 26, 39, and 40) that retained water during the fall of 2022. Two transects were surveyed using a Smith-Root POW Electrofisher backpack shocker (Model 15-D) with pulsed direct current along the long axis of the pool until a maximum of 30 minutes was reached. Fish were identified in the field using Rice and Meszaros (2014) and Trautman (1981). Reptile presence was noted; however, standardized collection methods were not utilized during this initial survey.

RESULTS

Our initial survey of the newly constructed pools at Oakwoods Nature Preserve yielded over 23,000 bacterial taxa (see Supplemental Material, **[TINYURL HERE]**), 81 algal genera (Table 1), and 51 macroinvertebrate taxa (Table 2), in addition to hylid and ranid frogs, common garter snake, green sunfish, common carp, golden shiner, and bluntnose minnow (Table 3).

23,802 different bacterial taxa were identified. Dominant phyla included Proteobacteria, Acidobacteria, Verrucomicrobia, Actinobacteria, Chloroflexi, Planctomycetes, Firmicutes, and Nitrospirae. Several algal genera were reported from 90% of the pools, including *Achnanthidium*, *Anomoeoneis*, *Caloneis*, *Craticula*, *Fragilaria*, *Geissleria*, *Jaaginema*, *Navicula*, *Neidium*, *Nitzschia*, *Pinnularia*, *Pseudofallacia*, *Rossithidium*, *Surirella*, and *Tryblionella*. Ten macroinvertebrate taxa were found in over 50% of the pools at Oakwoods, including Belostomatidae, Chironomidae, Copepoda, Corixidae, Daphniidae, Dytiscidae, Haliplidae, Hydrophilidae, Ostracoda, and Physidae.

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Table 1. List of algal genera from periphyton and macroalgal surveys of Oakwoods Nature Preserve wetland complex. *Indicates that the genus was part of the periphyton and macroalgal communities.

Cyanobacteria	*Anabeana*
	Chroococcus
	Gleocapsa
	Gleothece
	Jaaginema
	Oscillatoria
	Phormidium
	Plectonema
	Synechocystis
Chrysophytes	*Ochramonas*
Diatoms	*Achnanthes*
	Achnanthidium
	Amphipleura
	Amphora
	Anomoeoneis
	Brachysira
	Caloneis
	Cocconeis
	Craticula
	Ctenophora
	Cyclotella
	Cymatopleura
	Cymbella
	Cymbopleura
	Denticula
	Diadesmis
	Diatoma
	Diploneis
	Encyonema
	Fragilaria
	Fragilariforma
	Frustulia
	Geissleria
	Gomphoneis
	Gomphonema
	Gomphosinica

Diatoms *continued*	*Gomphosphenia*
	Gyrosigma
	Hantzschia
	Hippodonta
	Luticola
	Melosira
	Meridion
	Microfissurata
	Navicula
	Neidium
	Nitzschia
	Pinnularia
	Placoneis
	Planothidium
	Pleurosigma
	Psammothidium
	Pseudofallacia
	Reimeria
	Rhoicosphenia
	Rhopalodia
	Rossithidium
	Sellaphora
	Stauroneis
	Surirella
	Synedra
	Tryblionella
	Ulnaria
Dinoflagellates	*Ceratium*
	Gymnodinium
Euglenoids	*Euglena*
	Phacus
	Trachelomonas
Green Algae	*Akistrodesmus*
	Chlamydomonas
	Chlorella
	*Cladophora**
	Closterium
	Cosmarium
	Micrasterias
	*Microspora**
	*Mougeotia**
	*Oedogonium**
	*Pithophora**
	Scenedesmus
	*Spyrogyra**
Green Algae *continued*	*Staurastrum*
	Stichococcus
Xanthophytes	*Vaucheria**

Table 2. List of macroinvertebrate taxa collected from Oakwoods Nature Preserve wetland complex. Macroinvertebrates were identified to family (occasionally genus) for all insects and molluscs, and to order for annelids and non-arthropod insects.

Mollusca	Physidae
	Planorbidae
	Sphaeriidae
Annelida	Hirudinea
	Oligochaeta
Arthropoda (non-insects)	Amphipoda
	Cambaridae
	Copepoda
	Daphniidae
	Hydracarina
	Isotomidae
	Ostracoda
	Pisauridae
Arthropoda (insects)	**Ephemeroptera**
	Ameletidae
	Baetidae
	Caenidae
	Ephemeridae
	Isonychiidae
	Siphlonuridae
	Tricorythidae
	Plecoptera
	Perlidae
	Odonata
	Aeshnidae
	Calopterygidae
	Coenagrionidae
	Gomphidae
	Lestidae
	Libellulidae
	Hemiptera (Suborder Heteroptera)
	Belostomatidae
	Corixidae
	Gerridae
	Mesoveliidae
	Notonectidae
	Veliidae

Arthropoda (insects) *continued*	**Trichoptera**	
		Odontoceridae
		Polycentropodidae
	Coleoptera	
		Dryopidae
		Dytiscidae
		Gyrinidae
		Haliplidae
		Hydrophilidae (Berosus)
		Hydrophilidae (Laccobius)
		Noteridae
	Diptera	
		Ceratopogonidae
		Chaoboridae
		Chironomidae
		Culicidae
		Dixidae
		Ephydridae
		Stratiomyidae
		Tabanidae
		Tipulidae

Table 3. Vertebrate species collected from Oakwoods Nature Preserve wetland complex.

Fish	**Centrarchidae**	
		Green sunfish (*Lepomis cyanellus* Rafinesque)
	Cyprinidae	
		Common carp (*Cyprinus carpio* L.)
		Bluntnose minnow (*Pimephales notatus* Rafinesque)
		Golden shiner (*Notemigonus crysoleucas* Mitchill)
Amphibians	**Ranidae**	
		American bullfrog (*Lithobates catesbeianus* Shaw)
		Green frog (*Lithobates clamitans* Latreille)
		Northern leopard frog (*Lithobates pipiens* Schreber)
		Western chorus frog (*Pseudacris triseriata* Wied-Neuwied)
Reptiles	**Colubridae**	
		Eastern garter snake (*Thamnophis sirtalis* L.)

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