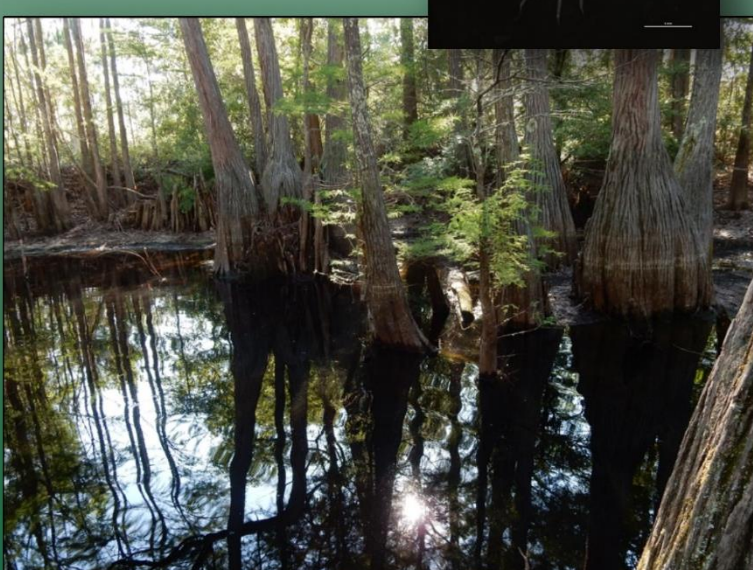
An Evaluation of Amphibian and Macroinvertebrate Communities in North Carolina Wetlands with Regards to Restoration Techniques

A Report to the EPA
by the North Carolina Division of Water Resources
Water Sciences Section
December 2016



AN EVALUATION OF AMPHIBIAN AND MACROINVERTEBRATE COMMUNITIES IN NORTH CAROLINA WETLANDS WITH REGARDS TO RESTORATION TECHNIQUES

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North Carolina Wildlife Resources Commission

December 2016

Citation: Gianopulos, K., V. Baker, and G. Rubino. 2016. An evaluation of amphibian and macroinvertebrate communities of North Carolina wetlands with regards to restoration techniques. Final Report to the EPA, Region IV. Funded by grant CD-00D01512. 173pp.

Acknowledgements

Any project of this magnitude involves a large number of people, from planners to data collectors to data analysts to writers to reviewers. This project is no exception. It was made possible through funding from the EPA's Wetland Program Development Grant program (CD-00D01512). Ross Vander Vorste and Virginia Baker conceived the idea and planned the project. Gregory Rubino and Larry Eaton assisted Virginia Baker as the primary field team, with additional data collection help from James Graham, Anthony Scarbraugh, Lori Montgomery, Michael Coleman, and Kristie Gianopulos. North Carolina Wildlife Resource Commission staff Jeff Hall, Jeff Humphries, and Nathan Shepard selected many sites and collected amphibian data. Many thanks to Jeff Humphries for listening to hours and hours of frog calling recordings. Kristie Gianopulos analyzed the data and authored the report, and Virginia Baker, Amanda Mueller, Ross Vander Vorste, Larry Eaton, Greg Rubino, and Cyndi Karoly provided valuable reviews.

Photo credits on cover page clockwise from top as follows: Brandon's Pond site (Kristie Gianopulos), Southern Leopard Frog (*Rana sphenocephala*) (Kristie Gianopulos), Little Little Dismal Pond site (Jeff Humphries), Cypress Pond site (Kristie Gianopulos), *Crangonyx* sp. (BIO Photography Group, Biodiversity Institute of Ontario), Dover Pond site (Kristie Gianopulos).

"But why worry about the plants and animals and insects? Because if they thrive, then it is healthy for people, too. If the water is dirty, the fish are rotting, the vegetation is dying, it is not safe for humans."
-- Gail Fishman, author of *Journeys Through Paradise: Pioneering Naturalists in the Southeast*

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Executive Summary

Aquatic biota such as amphibians and macroinvertebrates are a vital to every wetland ecosystem, as they provide important links between lower and upper trophic levels. Many macroinvertebrates convert organic plant material into animal biomass, which in turn feeds larval amphibians, aquatic insects, fish, and birds. Economically important fish and birds depend on wetland amphibians and macroinvertebrates for food. Yet, wetland restoration practices in North Carolina do not necessarily insure replacement of lost habitat for aquatic biota.

This project was an effort to understand these wetland-dependent animal communities in natural wetlands as well as in restored wetlands as a step toward better mitigation design and restoration practices. Wetlands that were restored through re-establishment of hydrology and vegetation contained greater numbers of amphibian and macroinvertebrate taxa than closed canopy reference wetlands. These re-establishment sites generally had permanent water, whereas most of the closed canopy reference sites dried out during the study. The re-establishment sites were also still in transition between open canopy and closed canopy systems and may decrease in amphibian and macroinvertebrate diversity as they mature. Wetlands that were restored (enhanced) by removal of canopy trees from historically open wetlands were successful in reaching amphibian and macroinvertebrate utilization comparable to open canopy reference sites.

Open canopy wetlands (reference and enhanced sites) provided better quality habitat for macroinvertebrates and amphibians than closed canopy reference or re-establishment sites. Our findings indicated that the highest ranked wetland sites for macroinvertebrates and amphibians were open canopy marsh-type wetlands containing aquatic plants, moderate cover by emergent vegetation, very few large trees, and no fish. Dissolved oxygen was high and specific conductivity was low in these wetlands. Closed canopy (forested) wetlands were not utilized as heavily by amphibians or macroinvertebrates as open canopy systems. The two state-listed threatened species found in this study (tiger salamander [*Ambystoma tigrinum*] and Carolina gopher frog [*Rana capito*]) were only found on open canopy wetlands. To improve habitat potential for aquatic biota on mitigation sites, mitigation design should incorporate a mosaic of habitat types that includes open canopy marsh areas disconnected from permanent water to maintain seasonally drying, fish-free habitat.

Introduction

Aquatic biota such as amphibians and macroinvertebrates are a vital to wetland ecosystems, as they provide important links between lower and upper trophic levels. Many macroinvertebrates convert organic plant material into animal biomass, which in turn feeds larval amphibians, aquatic insects, fish, and birds. Economically important fish, birds, and mammals depend on wetland amphibians and macroinvertebrates for food.

Wetlands also provide other important functions that benefit humans such as water quality improvement, flood control, and groundwater replenishment. In North Carolina, as across the nation, dredge and fill impacts to wetlands must be mitigated for to offset loss of wetland functions. Mitigation can be done by creating new wetlands, preserving existing wetlands, enhancing functions of existing wetlands, re-establishing former wetlands, or rehabilitating degraded wetlands. Each of these methods of mitigation carries its own level of credit to compensate for losses; re-establishment of former wetlands yields the most mitigation credit per acre, followed in decreasing order by rehabilitation, enhancement, creation, and preservation of existing wetlands. Once a mitigation site is completed on the ground, a period of monitoring is required (usually 5-7 years in North Carolina), to ensure that the goals of the mitigation activity are accomplished. Freshwater wetland mitigation success criteria in North Carolina are based solely on hydrology and vegetation success, while habitat for aquatic biota are not included.

The primary means of wetland mitigation in North Carolina is using re-establishment to restore former wetlands. The EPA defines wetland restoration by re-establishment as "the manipulation of the physical, chemical, or biological characteristics of a site with the goal of returning natural/historic functions to a former aquatic resource. Re-establishment results in rebuilding a former aquatic resource and results in a gain in aquatic resource area and functions." (40 CFR Part 230). Wetland re-establishment is often done in North Carolina for mitigation credit to offset permitted impacts elsewhere. Re-establishment first involves restoring altered hydrology of an area that was formerly wetland, often by plugging or eliminating drainage ditches. Secondly, upland vegetation is often removed and replaced by planting wetland trees.

Wetland enhancement is another means of gaining mitigation credit. The focus of this technique is improving certain wetland functions. The EPA defines wetland enhancement as "the manipulation of the physical, chemical, or biological characteristics of an aquatic resource to heighten, intensify, or improve a specific aquatic resource function(s). Enhancement results in the gain of selected aquatic resource function(s), but may also lead to a decline in other aquatic resource function(s). Enhancement does not result in a gain in aquatic resource area."

It is not well known in North Carolina how effectively wetland restoration practices replace or improve lost habitat for aquatic biota. A literature review on aquatic biota studies in North Carolina mitigation wetlands indicated that few studies have been conducted on amphibians (Petranka and Holbrook 2006) and none on aquatic macroinvertebrates in North Carolina wetland mitigation sites. During mitigation site monitoring, the inclusion of other biotic factors such as macroinvertebrate and amphibian information would help regulators better gauge how successful a given restoration project is in recovering or replacing lost wetland functions.

The purpose of this study was to assess two wetland restoration techniques (re-establishment and enhancement) with regards to amphibian and macroinvertebrate communities, by comparing those on restoration sites to those on reference sites. Re-establishment sites were wetlands that had been historically drained for silviculture or agriculture, and were restored for mitigation credit by restoring hydrology and replanting wetland tree species to re-establish the wetlands.

Enhancement sites were wetlands on lands under public land management, which were restored for the purposes of improving habitat for aquatic biota, and not for mitigation credit. These sites were historically open-canopy systems, but fire suppression had resulted in invasion by trees. The wetlands were enhanced using mechanical tree removal and/or burning to remove the canopy and return the wetlands to open-canopy systems to improve habitat conditions specifically for amphibian species (Thurgate and Pechmann 2007). The premise behind this type of restoration was that in closed canopy systems, the accumulation of organic matter causes a drop in pH and dissolved oxygen, which are difficult for amphibian species to tolerate (Smith and Braswell 1994; Babbitt et al. 2006). Routine fire is thought to be related to less acidic conditions in North Carolina wetlands. Canopy removal and burning eliminate organic material and lessen litter input, which improves habitat conditions for amphibian species.

Closed canopy reference wetlands and open canopy reference wetlands were sampled alongside the re-establishment and enhancement wetlands to more fully understand the biotic communities present in natural wetlands.

MACROINVERTEBRATES AS INDICATORS

Macroinvertebrates are foundational to food webs in aquatic ecosystems, and are important food sources for waterfowl and other birds, amphibians, fish, and some mammals. Macroinvertebrates have been found to be reliable indicators of environmental health of streams and lakes, and are now being used routinely in bioassessments of these waters. North Carolina utilizes a macroinvertebrate biotic index (MBI) to characterize streams. This index incorporates tolerance values for macroinvertebrate species, which are gauges of general pollution or stress tolerance, regardless of source (Lenat 1993).

In general, macroinvertebrates can be good indicators of environmental condition, particularly in streams or flowing water, because they are widely distributed, sensitive to variation in water quality (especially nutrients and metals), and exhibit different levels of tolerance for environmental stress, depending on the species (EPA 2002). They also have life cycles which are dependent on water for long periods of time, which means they can be present long enough to be exposed to environmental changes. However, they can be insensitive to some types of stress like herbicides. Natural factors, in addition to water quality, impact their distribution and abundance, so caution and expertise must be used in interpreting results, especially biotic index results.

Because wetlands often contain anoxic conditions and temporarily dry out, they can predominantly contain macroinvertebrates which are tolerant of these stressful conditions. This tendency makes the macroinvertebrate biotic index somewhat less useful in wetlands than in streams, where the index is used to assess pollution levels based on presence/absence of “sensitive” or “tolerant” macroinvertebrates. However, comparisons of overall macroinvertebrate community structure in natural wetlands with that of mitigation wetlands can shed light on whether mitigation wetlands are effectively replacing habitat for macroinvertebrates (Meyer and Whiles 2008). Although macroinvertebrates have not often been used in assessments of wetland health or wetland restoration success, interest in their usefulness as indicators in wetlands is growing around the world (Brown and Batzer 2001, Sharma and Rawat 2009, Marchetti et al. 2010, Epele and Miserendino 2015). More basic data on which macroinvertebrates occur in wetlands, particularly in North Carolina wetlands, is needed to uncover traits or species which could be good indicators of wetland condition.

AMPHIBIANS AS INDICATORS

The Southeast is home to a large number of amphibian species. North Carolina alone is home to over 95 species of amphibians and is known for its diversity of salamanders, boasting more than any other state in the Union with 54 species (Braswell 2006). Amphibians have complex life-cycles, dependent on both wetland and upland habitats. Deforestation and the increase of acidic conditions and pollutants such as nitrogen and heavy metals have adversely affected these environmentally sensitive species (US EPA 2002b; Smith and Braswell 1994; Sparling 2003; Wilson and Dorcas 2003). Amphibians have been used as indicators of water quality (Veselka 2008), restoration success (Walls et al. 2014a; Walls et al. 2014b), and wetland hydrology (Waddle 2006), although urbanization can interfere with use of amphibians as indicators of wetland integrity (Guzy et al. 2012).

Fire suppression has negative effects on amphibian communities, especially those in longleaf pine ecosystems. Lack of fire allows hardwood species to invade wetlands, close the canopy of formerly open canopy wetlands, and increase leaf litter, woody debris, and shrubs in the understory. Amphibian communities of hardwood forests and wetlands differ substantially from longleaf pine ecosystems. Frequent summer fires, on a nearly annual basis, mimic historically natural conditions and keep hardwood forests from invading (Means et al. 2004). Where controlled burning is not feasible, canopy removal can be performed manually as a restoration technique.

Because amphibian species vary in their sensitivity to environmental stress and changes in habitat condition, species composition in wetlands can yield valuable information. If particularly sensitive species are found, a wetland can be presumed to be of higher quality or in better condition than a wetland where only more tolerant species are found. However, *absence* of a particular species can have many potential causes, such as wetland degradation, migration barriers, population dynamics, or species rarity. Amphibian assemblages can be highly dynamic from year to year within a wetland (Hecnar and M'Closkey 1996), so longer term studies are imperative. Additionally, amphibians can be cryptic and difficult to find, so use of a variety of sampling methods is important, such as egg surveys, larval dipnetting, and auditory call surveys. The impacts of natural variations on species detection are lessened through longer-term sampling efforts at multiple locations, and with visual as well as auditory data collection.

Methods

SITE SELECTION

This project involved data collection on 16 wetland study sites of four different types; 4 re-establishment sites, 4 enhancement sites, and 8 reference sites (4 open canopy and 4 closed canopy) (Figure 1) (Table 1). The expected endpoint of re-establishment sites was closed canopy wetlands, so re-establishment sites were compared to closed canopy wetlands. These re-establishment sites were restored for non-riverine mitigation credits. The desired outcome for enhancement sites was open canopy wetlands, so the enhancement sites were compared to open canopy wetlands. All sites were located in the Coastal Plain and Sandhills ecoregions of North Carolina to minimize ecoregional differences (Figure 2). Site selection was based on availability, access, best professional judgment, location, wetland type, and individual site conditions. All site wetlands (or wetland sections) were delineated using the established 1987 Corps of Engineers Wetland Delineation Manual (US ACE 1987). Amphibians, aquatic macroinvertebrates, hydrology, water quality, vegetation characteristics, and Level 1 and Level 2 assessments were sampled at each site. Specific details on methodology, replicates, parameters of interest, and sample period are discussed later in this section.

Re-establishment Sites - Re-establishment sites were identified through review of the NC Division of Water Resources (DWR) mitigation database, and communication with other NC DWR regulatory staff, NC Wildlife Resources Commission (NC WRC) staff, NCDEQ Division of mitigation Services managers (formerly called the Ecosystem Enhancement Program), and other mitigation provider managers. The NC DWR mitigation database, along with follow-up communication, was used to identify non-riparian wetland re-establishment sites that were ≥ 5-16 years old, met hydrology and vegetation success criteria, and were located in the NC coastal plain or sandhills region. NC DWR mitigation files, monitoring plans and reports, NC digital orthoimagery, and Google maps were used to do a desktop review of potential re-establishment sites. Carolina bays and sites that appeared to have ponded water conditions were chosen for reconnaissance. Best professional judgment by scientist from NC DWR and NC WRC was used to choose sites that appeared to have conditions that could house aquatic biota. These sites were all compensatory mitigation sites that generated mitigation credit through restoration by re-establishment of hydrology and wetland vegetation (primarily with ditch plugging and planting). Because the chosen re-establishment sites were all very large, a section of each re-establishment site (0.7 to 3.5 acres in size) was chosen for study through best professional judgment and delineated for assessment.

Enhancement Sites - Enhancement sites were selected by NC WRC herpetology staff using digital orthoimagery, USGS maps, historical aerials and records. The enhancement sites were non-riparian mineral based Carolina bays and depressional basin wetlands located on NC WRC property. NC WRC staff speculated that these forested wetlands were historically open-canopied whose surfaces were a mosaic of open water, herbaceous emergent plants, and aquatic vegetation maintained with a natural fire regime. Open canopy ponded wetlands that are unconnected to overland flow tend to be fish-free, less acidic due to lack of tree leaves, hold water more months out of the year since evapotranspiration through herbaceous vegetation occurs at a much slower rate than through trees, and provide herbaceous vegetation structure for aquatic fauna habitat. These open canopy site characteristics have the potential to attract aquatic biota. The NC WRC wetland managers applied a variety of enhancement techniques to thin and remove canopy trees to restore these historic open-canopy conditions (1-3 years prior to this study). Techniques included hand cutting, root bowl and duff removal via scraping with a bulldozer, woody debris removal, and seasonal prescribed fires.

Open and Closed Canopy Reference Sites - The open and closed canopy reference sites were non-riparian mineral based Carolina Bays or depressional basin wetlands. NC WRC staff used best professional judgment, location, and availability of access to choose the reference sites. Tree cover for open canopy sites was less than 20 percent. Tree cover on closed canopy sites was greater than 60 percent.

Figure 1. Locations of study sites (16) in North Carolina.

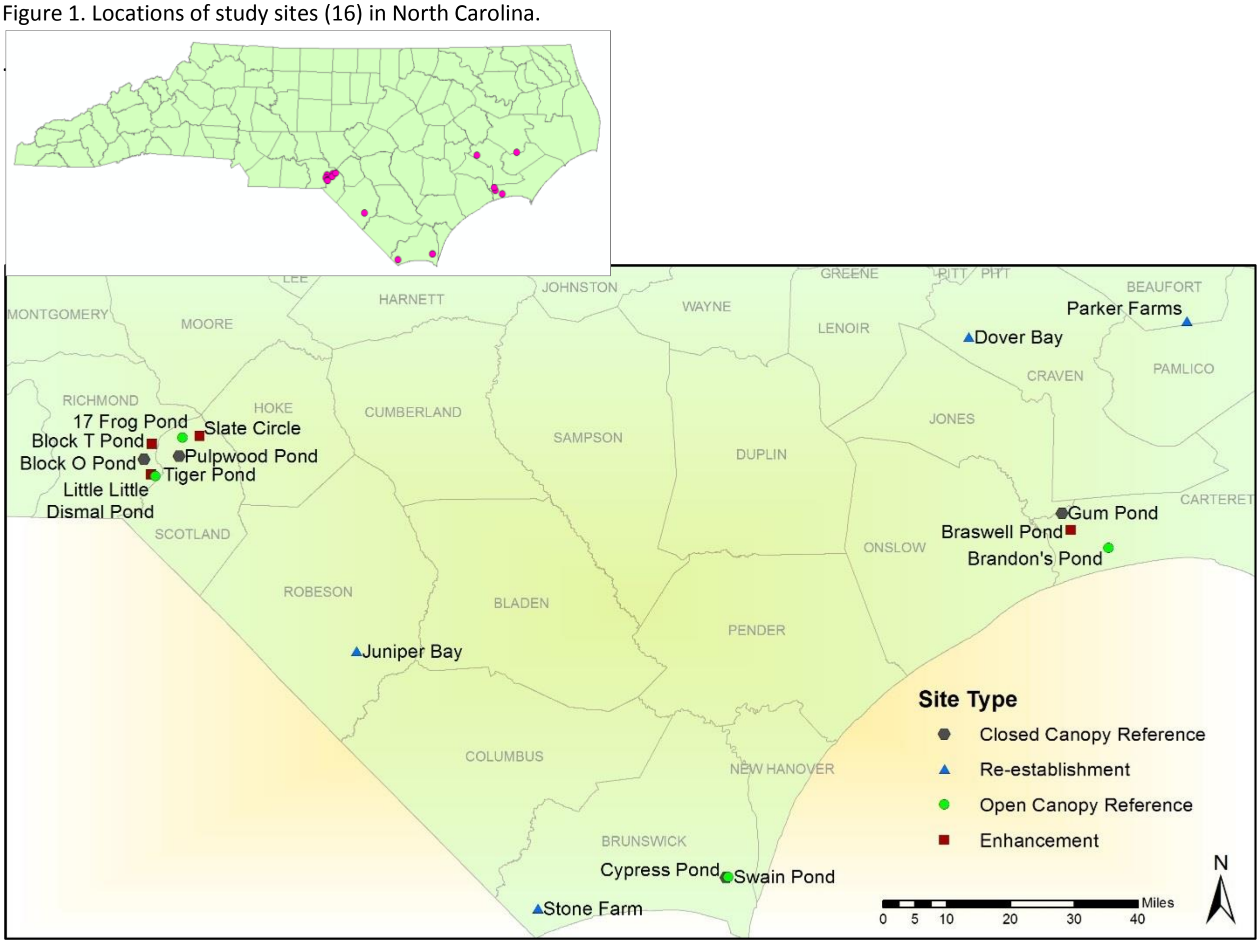


Figure 2. North Carolina ecoregions. (Source: city-data.com).

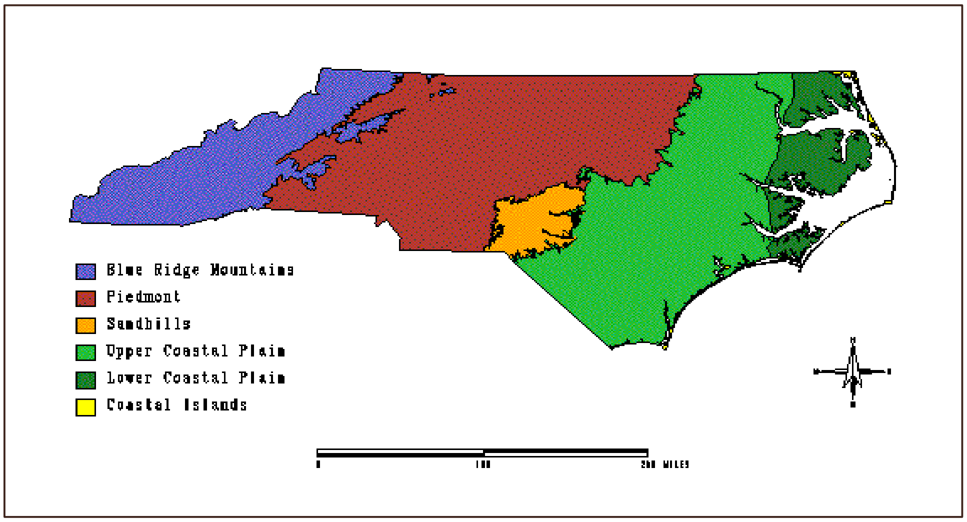


Table 1. Basic site characteristics of chosen study sites.

Site Type	Site Name	County	Assessment Area Size (ac)	NCWAM Wetland Type	Latitude/Longitude
Closed Canopy Reference	Block O Pond	Richmond	1.97	Basin	34.939958, -79.616591
	Cypress Pond	Brunswick	0.16	Basin	33.987090, -78.020750
	Gum Pond	Carteret	1.18	Non-Riverine Swamp Forest	34.803927, -77.084014
	Pulpwood Pond	Scotland	0.62	Basin	34.948245, -79.519612
Re-establishment	Dover Bay	Craven	2.63	Non-tidal Freshwater Marsh (within a Carolina Bay)	35.208541, -77.333306
	Juniper Bay	Robeson	3.51	Pine Flat (within a Carolina Bay)	34.505791, -79.028506
	Parker Farms	Beaufort	1.81	Hardwood Flat	35.234486, -76.728531
	Stone Farm	Brunswick	0.69	Non-tidal Freshwater Marsh (within a Carolina Bay)	33.919360, -78.534507
Open Canopy Reference	17 Frog Pond	Scotland	4.27	Basin	34.989864, -79.510854
	Brandon's Pond	Carteret	2.54	Basin	34.723229, -76.959074
	Swain Pond	Brunswick	0.38	Basin	33.987731, -78.015106
	Tiger Pond	Richmond	0.39	Basin	34.902135, -79.585019
Enhancement	Block T Pond	Richmond	12.06	Basin	34.974839, -79.594852
	Braswell Ponds	Carteret	0.097	Basin	34.765363, -77.061424
	Little Little Dismal Pond	Richmond	0.50	Basin	34.906189, -79.597010
	Slate Circle	Scotland	0.89	Basin	34.994400, -79.463500

SITE DESCRIPTIONS

Closed Canopy Reference Wetlands

Aerial photographs and on-site photographs for the closed canopy reference sites are presented in Figure 3. Topographic maps for each site are located in Appendix A and additional photographs taken throughout the study are in Appendix B.

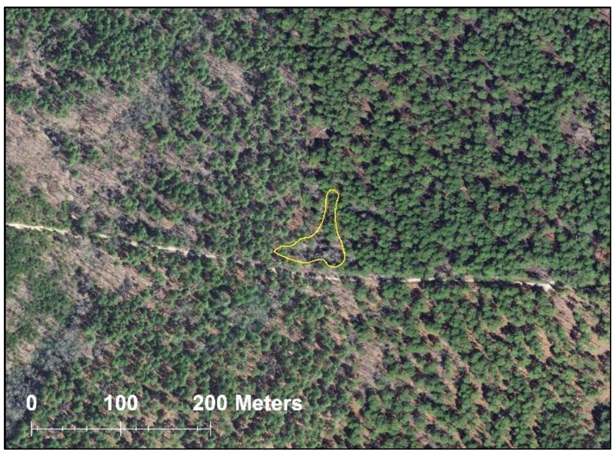
Figure 3. Aerial photography and on-site photographs of each closed canopy reference site.

BLOCK O POND ---

CYPRESS POND ---

GUM POND --

PULPWOOD POND --



<u>Block O Pond</u>: Block O site was located 3.7 kilometers south of Rockingham Speedway (motor race track) in Hoffman, Scotland County. It was 0.27 kilometers from paved road and railroad tracks and was found at an elevation of 125.9 meters. It was located within the Sandhills Gameland managed by the WRC. The physiographic setting was the Sandhills region of North Carolina, part of the upper Lumber River Basin and mapped as Tertiary and Cretaceous deposits of the Pinehurst and Middendorf formations. Medium to coarse sand was the predominant deposit type with varying mixtures of silt or clay. The Sandhills exhibit medium to high relief and dissection with local streams.

The site lies 36.6 meters higher in elevation than the nearest named water body, McLeod's Lake. An active sand and gravel mine was located 0.49 kilometers to the east. The surrounding upland was covered by a dry longleaf pineland community, with the pine overstory dominating scrubby oak shrubs and grass herb layer. The top layer of soil in this surrounding upland was extremely sandy and humus deficient. Soils surrounding the pool and wetland were mapped as the upland Wakulla and Candor excessively drained sands. The wetland was underlain by Johnson muck or possibly a modified Bibb component.

The delineated wetland at the site was 1.97 acres in extent. It was completely surrounded by upland and at approximately the same elevation. A ditch up to two meters in depth runs northwest-southeast into the center of the wetland area. Though the site was actively managed by the WRC, natural fire has likely been excluded from the wetland for many decades based on the presence of hardwoods. Prescribed burning had not been conducted at this site in recently known history. This pond represented most isolated wetlands that have been excluded from fire and/or ditched in the Sandhills region (WRC pers. comm. 2016).

This wetland seasonally flooded up to 0.5 meter in depth. At the time of the study, trees were mostly sweetgum (*Liquidambar styraciflua*), black gum (*Nyssa sylvatica*), and red maple (Acer rubrum). Swamp titi (*Cyrilla racemiflora*) and highbush blueberry (*Vaccinium corymbosum*) made up the shrub layer.

The NC Wetland Assessment Method (NCWAM) (NC Div. of Water Resources 2008) rapid assessment classification for Block O was basin-type wetland while the NC Natural Heritage Program (NC NHP) classification for the site was vernal pool. The ground surface within the wetland was relatively shallow and concave. The ditch, large logs, vegetation stem clumps, and large tree roots created a high degree of microtopography.

<u>Cypress Pond</u>: The Cypress Pond site was located on the western border of the Military Ocean Terminal at Sunny Point (MOTSP) facility in Brunswick County, in the extreme southeastern corner of North Carolina. It was 0.13 kilometers north of the MOTSP paved two-lane access road and adjacent to a single-track railroad. It lies approximately 6.3 kilometers northeast of the Cape Fear River and was at approximately the same elevation as the nearby access road (10.6 meters above sea level). There were two large ponds formed from defunct sand mining operations immediately to the east and southeast. The land was owned by the WRC, but there was currently no active management, including intentional burning, being practiced at the site.

The site was geomorphically considered to be a limesink formation, where the topographic low that contains the wetland was formed from dissolution of underlying shelly Quarternary deposits and limestone of the Castle Hayne units. The site was located within a mapped Mandarin fine sand unit.

The surrounding upland area was dominated by loblolly pines and scrub oak, reminiscent of turkey oak scrub areas of the Sandhills region to the west. The wetland area was a very small (0.165 acre) but very deep bowl-shaped depression with marked relief of two to four meters between the immediate upland elevation and the bottom of the wetland pond. It was perennially flooded. There was notable microtopography in both the uplands and outer third of the wetland, due to mature tree hammocks, roots and debris. Mature pond cypress trees had established in the outer third but not in the center due to the water depth. Bladderwort (*Utricularia* sp.), an insectivorous aquatic plant, was also common throughout.

The NCWAM classification of Cypress Pond was basin-type wetland while the NC NHP designation was permanently wet depression pond.

<u>Gum Pond</u>: The Gum Pond site was located 4.6 kilometers east of Route 58 in Carteret County within the Pocosin Wilderness of the Croatan National Forest, in the far eastern part of the state. It was 30 meters west of Forest Road 144, a one-lane gravel road. It was on the edge of the floodplain of Hadnot Creek, a tributary to the White Oak River, over 6 kilometers to the southwest. The nearest large waterbody was Great Lake, 6.3 kilometers to the northeast. Ground surface at Gum Pond was about 9.1 meters above sea level.

The upland area around Gum Pond consisted of coastal plain pine flat forest, chiefly mature loblolly pines with scrubby oak and grass understory. There was little topographic relief within kilometers of the site, including along the upland/wetland boundary.

According to NC WRC staff, the wetland site had not been managed with prescribed fire for many decades. The surrounding uplands were thinned and burned at the beginning of this study.

The wetland itself was 1.18 acres in extent with hardwoods and other deciduous trees and shrubs dominating the vegetation. Red maple and swamp tupelo (*Nyssa biflora*) were common in the canopy, and there was a dense shrub layer of swamp titi and fetterbush lyonia (*Lyonia lucida*). Mapped soil across both the wetland and upland was Onslow loamy fine sand with Rains inclusions. There was a thick (0.7 meter) organic layer at the top of the soil profile in the wetland that includes large woody debris.

The wetland interior ground surface wasan extremely hummocky and pooled tangle of debris and roots of large shrubs and the mature hardwoods. Pool and hummock microtopographic relief reached over a meter in scale. The site was seasonally flooded with most pooled areas 0.3 meter deep (some deeper). This wetland consisted of many interconnected pools, rather than one large inundated area like most other sites. The NCWAM designation of Gum Pond was basin-type wetland and NC NHP classification was permanently wet depression pond.

Pulpwood Pond: The Pulpwood pond site was located within the Sandhills Gameland of Scotland County, east of Marston, NC. It was 3.5 kilometers northeast of Catawba Lake, 610 meters west of Hoffman Road, a two-lane unimproved sandy track, and between Jordan and Juniper Creeks. It was within the Lumber River Basin system and the Sandhills physiographic area. The site lies at 107 meters in elevation.

The site was located within an oak-hickory forest complex. The area immediately surrounding the pond and wetland was covered in a mature canopy of hardwoods and pine, an overstory that dominates scattered or patchy herbs and shrubs. Oak-hickory forests in the Sandhills region were known to occur in "fire shadows", places where fires burn unevenly or poorly due to high humidity and relatively sparse herb and shrub layers (Sorrie 2011). The area around Pulpwood Pond was actively managed with prescribed fires as recently as spring 2015. The upland forms a semicircular bowl or ridge surrounding the wetland to a relief maximum of approximately 9 meters.

The wetland area was 0.62 acres and was reminiscent or a continuation of the oak-hickory forest surrounding it, with very mature trees dominating a very light shrub and herb layer. The differential speciation of hardwoods inside the wetland area versus pine trees in the surrounding uplands suggests natural and prescribed fires either being excluded from the area or burning only when the ponded area contained water (WRC pers. comm. 2016). The ground surface within the wetland was very shallow, flat and uniformly concave. There was very little microtopography other than what tree roots provide.

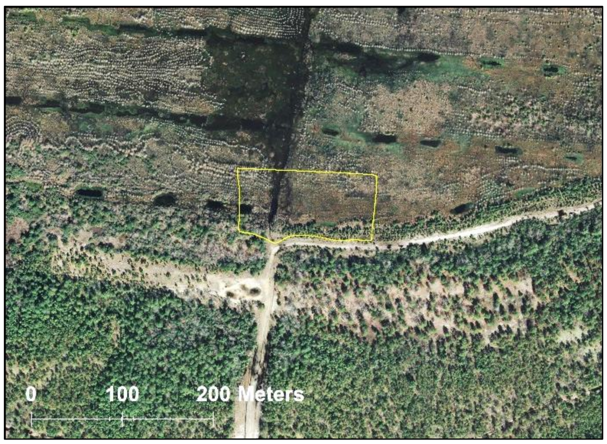
Mapped soils in the area include Ailey loamy sand and Pelion loamy sand. Soil in the wetland does not match the hydric components usually associated with these upland soils and indicates a deeper stratum has been exposed relative to the surrounding surficial layers. A limiting layer of greenish gray sandy clay was encountered from 84 centimeters to 91 centimeters deep. The wetland was considered to be a NCWAM basin type and a NC NHP vernal pool.

Re-establishment Wetlands

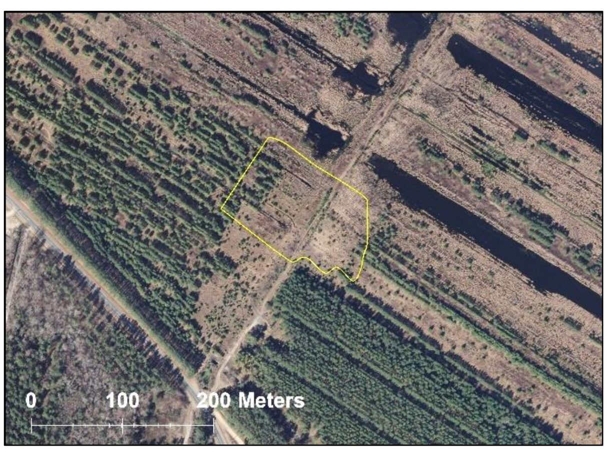
Aerial photographs and on-site photographs for the wetland re-establishment sites are presented in Figure 4. Larger aerial contexts for individual sites are shown in Figures 5-8, because the re-establishment wetland sites were all quite large. A smaller representative assessment area (shown in Figure 4) was delineated for study within each re-establishment site. Topographic maps for each site are located in Appendix A and additional photographs taken throughout the study are in Appendix B.

Figure 4. Aerial photography and on-site photographs of each wetland re-establishment site.

DOVER BAY ---



JUNIPER BAY --



PARKER FARMS ---

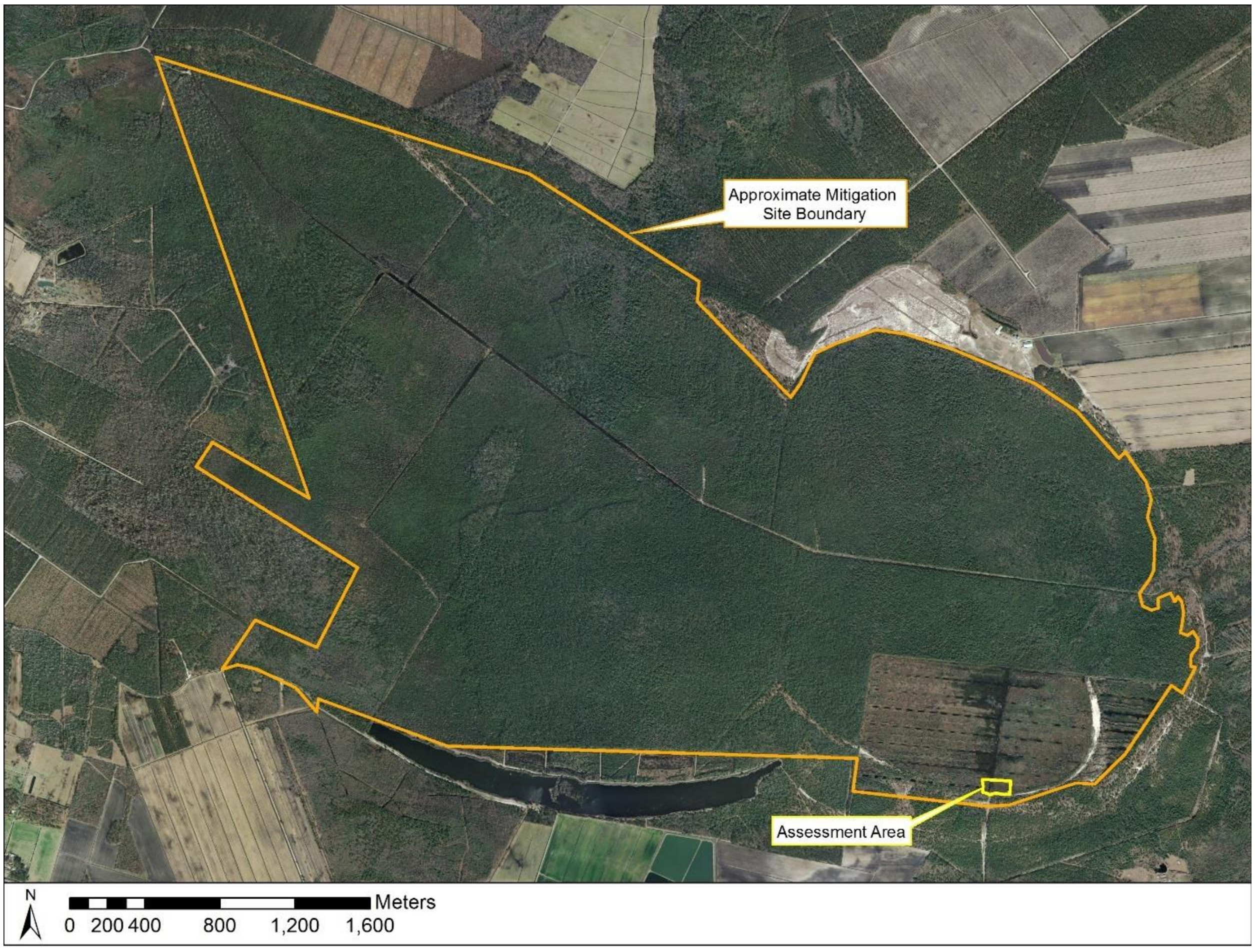
STONE FARM ---

Dover Bay: Dover Bay was a NC DWR wetland re-establishment site formed within a double (merged) Carolina bay formation located 9.7 kilometers southwest of the Neuse River main stem in Craven County and 20.9 kilometers southeast of Kinston, NC (Figure 5). The nearest road was old U.S. Highway 70, a paved two-lane artery between Kinston and New Bern 1.9 kilometers to the south. The bay(s) includes 7,008 acres of pocosin wetland that had been extensively ditched and logged in the past two centuries. They are headwaters to two tributaries to the Neuse River (Mosely Creek and Mill Branch).

This wetland re-establishment site was a permittee-responsible, off-site mitigation project for impacts from development of the NC Global Transpark, a large cargo transfer hub located in Kinston, NC. In the 1970s, this wetland had been mined for peat, which removed the historic organic horizon and is believed to have lowered the elevation of the original Carolina Bay wetland. A sand mine was added in the 1970s in the southewest side of the bay (Figure 5). A road and ditch network was added in the late 1970s and early 1980s, and a third of the bay was also logged.

Restoration (where the assessment area was located) and enhancement activities, including tree planting, began at the site in 1999, when the site was burned to get rid of upland species and the drainage network was plugged. In 2001, pond pine, loblolly bay, red bay, and pond cypress were planted with supplemental plantings occurring two years later. The re-establishment area comprises 2,971 total acres of the original bay.

Figure 5. Large-scale aerial photograph of the Dover Bay wetland re-establishment site.



The study assessment area was located in the southeast side of the re-establishment site, and included 2.63 acres of mainly open canopy wetland adjacent to a permanently inundated large ditch at the southwest corner of the Bay (Figure 5). The ground surface was relatively flat, with several deeper depressions scattered in the soft substrate. The hydrology of the assessment area was a typical perched water table context common to pocosin wetlands in the southeast and the coastal plain of North Carolina. The ditch system had been plugged and adjacent sections have become open water ponds due to perennial flooding. Historical aerial photos indicate the site has never lost its surface water since the ditch system was deactivated.

This re-establishment site turned out wetter than the mitigation providers anticipated, perhaps partly due to the lower elevation from historic removal of the organic layer for peat mining. Unanticipated excess flooding slowed tree growth in some areas, including a majority of the assessment area, leaving the site more open than a drier 10+ year old re-establishment site would normally be. At the time of the study, there was a highly dense herbaceous layer of sedges (*Carex* sp.) and rushes with some sphagnum (*Sphagnum* sp.) mixed in. Scattered planted cypress were surviving and red maple was starting to colonize some of the drier areas in the northern section of the assessment area. The historic road location had left a large ponded area in the middle of the assessment area. Most of the assessment area had water levels that were less than a foot deep, except in the old road bed (on the western edge of the assessment area) which was several feet deep.

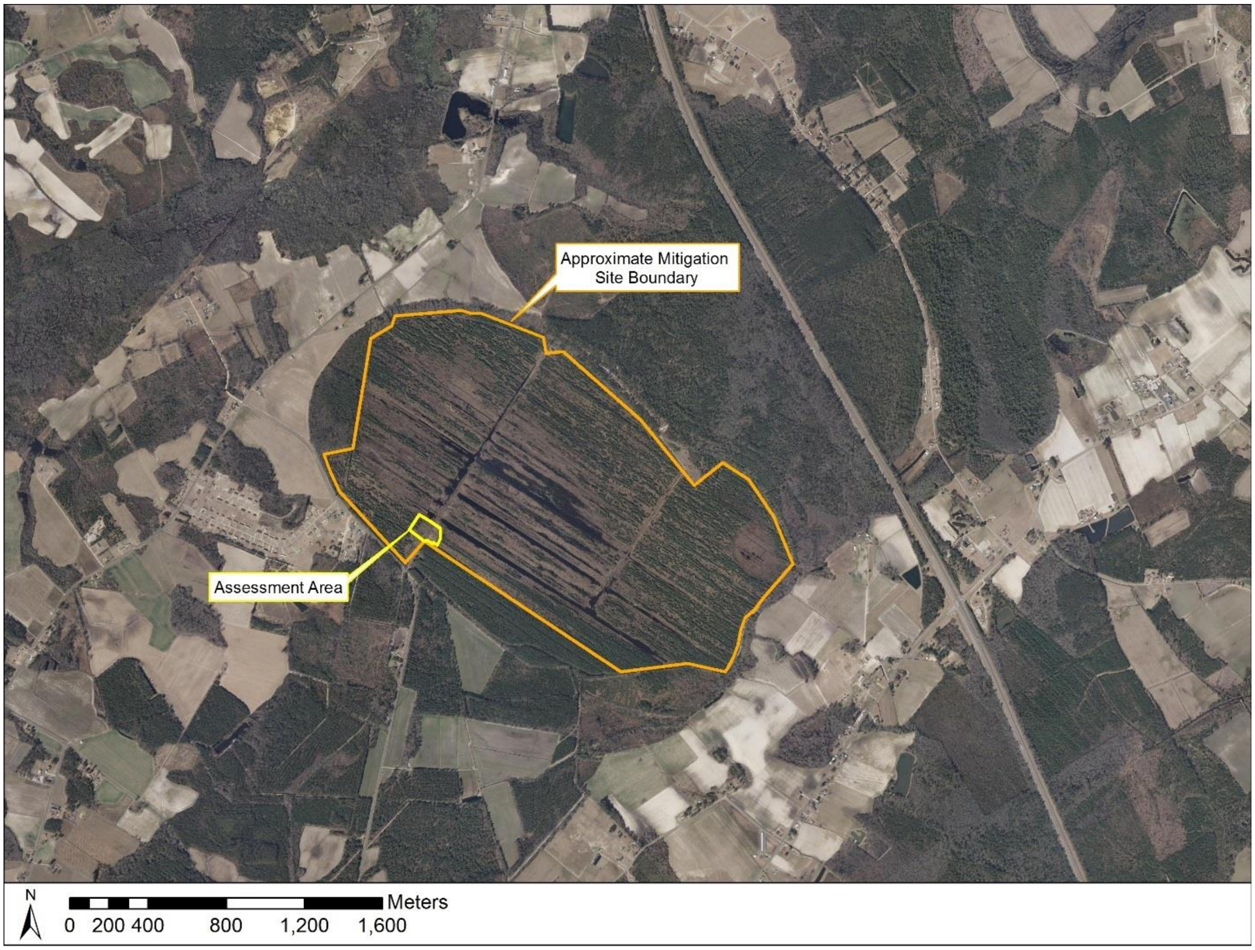
The soil of the assessment area was either Croatan muck overlying a Leon or Kureb sand variant or Murville mucky sand overlying an unmapped coarse sand to fine gravel layer or lens. There was an exposed bay rim of Kureb sand to the south and east of the wetland/upland border. The NCWAM classification for the Dover Bay assessment area was a non-tidal freshwater marsh. The NC NHP classification was a coastal plain semi-permanent impoundment.

<u>Juniper Bay</u>: The Juniper Bay re-establishment site was located 7.2 kilometers east of Fairmount, Robeson County, 12.54 kilometers south of Lumberton and 2.4 kilometers west of a four-lane paved highway (Route 74). The study area was located within a 728 acre double-formation Carolina bay within the Coastal Plain (Figure 6). The bay most likely historically contained pocosin natural communities but was extensively ditched, logged, and replanted between 1966 and 2000, and was used for various agricultural purposes, including longleaf pine tree production. Active agricultural activities ended in 1999 when Juniper Bay was acquired by the NC Department of Transportation for mitigation purposes to offset roadway impacts in the Lumber Basin. Wetland restoration efforts targeted 568 acres of the bay with the goal of re-establishing a peatland Atlantic white cedar forest and a pond pine woodland bay forest. Restoration work was done in two phases, in 2004 and 2006, when the ditches were plugged and/or regraded and trees including white cedar, pond pine, water tupelo, sweet bay, loblolly pine and bald cypress were planted and fertilized. This re-establishment site was closed out in 2010.

Juniper Bay is situated on a western upper terrace of the Lumber River floodplain. It was 35.6 meters in elevation, comparable to the headwaters of the nearest named water body (Hog Swamp), 2.98 kilometers to the northwest. The 3.51 acres of assessment area were located in the upper western portion of the northwestern-southeastern trending bay (Figure 6). The surrounding vegetation included strips of extensive herbaceous cover alternating with bands of shrubs, saplings and small trees. Within the assessment area, the soil was drier and more shrubby (wax myrtle [*Myrica cerifera*] and saltbush [*Baccharis halimifolia*]) closer to the perimeter ditch. Further from the perimeter ditch, the site was dominated by dense herbaceous species (softrush [*Juncus effuses*] and wool grass [*Scirpus cyperinus*]).

Juniper Bay's interior was underlain by Ponzer muck soils, with an outer band of sandy hydric soils. Relict ditch and dirt roadway features occur throughout the study area. These (negative) features hold most of the surface water and alternate with flat and frequently dry ground surfaces. Water could be seen leaving the site year-round through deep ditches to the west; however historical photos show the overall bay to be perennially wet above ground surface since the internal ditch system was intentionally deactivated. The NCWAM classification of Juniper Bay was a modified pocosin or possibly pine flat. The closest NC NHP equivalent classification was wet pine flatwoods.

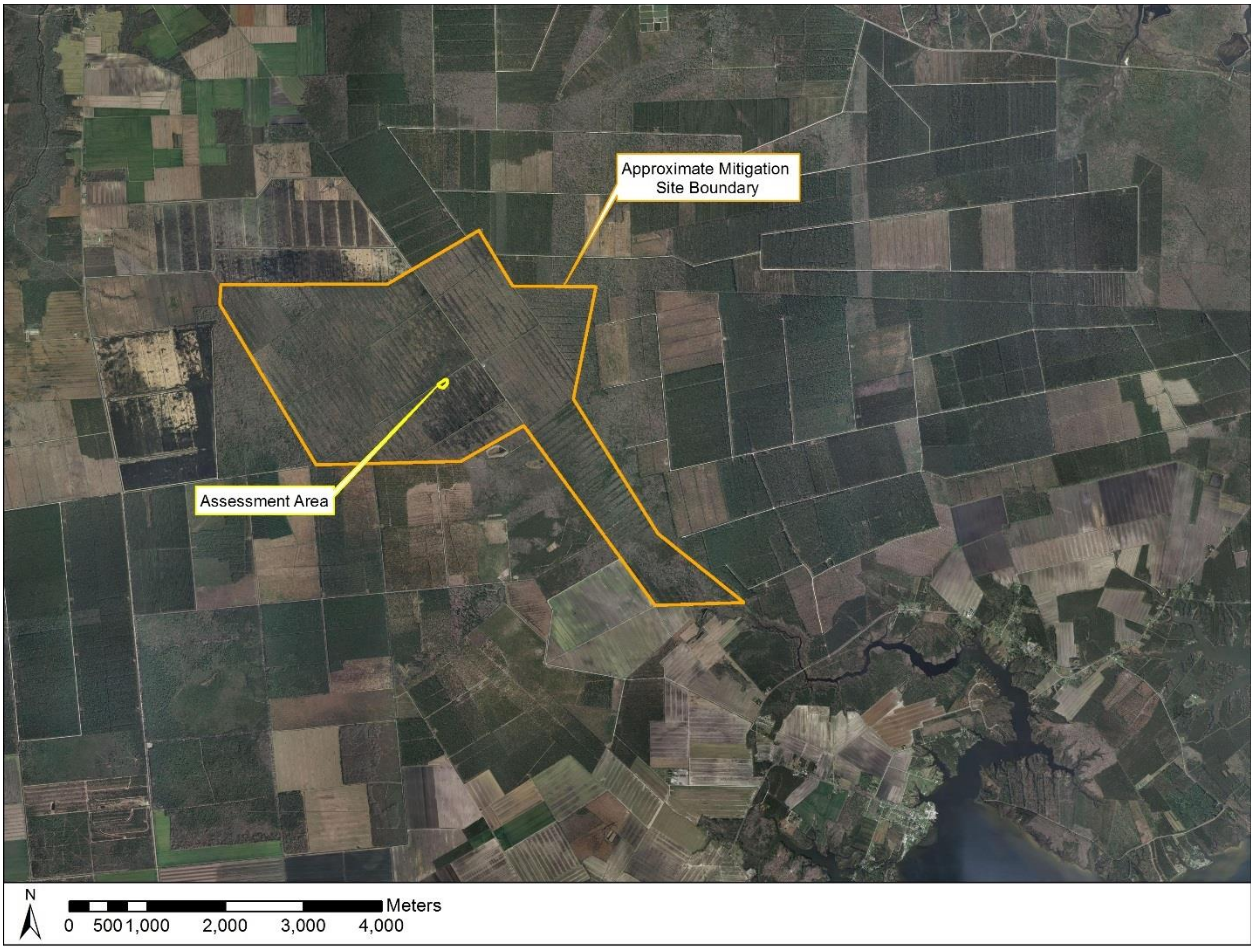
Figure 6. Large-scale aerial map of the Juniper Bay wetland re-establishment site.



Parker Farms: Parker Farms was a wetland re-establishment site constructed in 1997 to offset impacts from local mining operations near Aurora, NC, and encompasse 2,811 acres of wetland in southern Beaufort County in eastern North Carolina. This re-establishment site is a permittee-responsible mitigation site. Parker Farms lies approximately 16 kilometers west of Pamlico Sound and 4.4 kilometers northwest of Vandemere Creek, a tributary of Bay River. The greater wetland complex was immediately adjacent to brackish and salt marshes fringing Pamlico Sound (Figure 7). The site lies within the Neuse River basin.

The project assessment area was a 1.81 acre area located somewhat centrally within the re-establishment site. It was located on an inter-stream flat, likely historically composed of bald cypress, swamp tupelo, and red maple. The section where the assessment area was located was ditched, logged, and converted to cropland in the late 1970s and early 1980s. Restoration work began in 1997, when ditches and canals were plugged and spillways installed to drain off water that exceeded one foot in depth. Roads and ditches were used to separate sections of the mitigation site. The site was planted with six tree species. The site was closed out in 2001 after remedial planting for sections that had failed.

Figure 7. Large-scale aerial map of Parker Farms wetland re-establishment site.



The assessment area of Parker Farms had a closed canopy of bald cypress (Taxodium distichum), water tupelo (Nyssa aquatic), oaks (*Quercus* sp.) and willow trees (*Salix* sp.) that have thrived since planting. Additionally, other native herbaceous ferns, sedges and forbs and woody species (red maple) have volunteered. The herbaceous layer was patchy to sparse, and there was no shrub layer. The site was seasonally inundated with water generally less than 1 foot deep.

Elevation of Parker Farms was 2.7 meters to 3.5 meters above sea level and did not display relief within the entire re-establishment area. A network of extensive ditches surrounded the assessment area, at least some of which were drawing surface water toward Vandemere Creek. However, the saturated Ponzer muck soils, high local water table and (possible) local beaver activities allow for extensive and long-term ponding. The assessment area was separated from a large perennially ponded area to the northwest by a single lane gravel road. This section to the north was restored on a different timetable, and flooding is greater since it is adjacent to a canal. Extensive seasonal ponding also occurred to the south and southeast, associated with the assessment area.

The ground surface of the assessment area was fairly level and uniformly so. There was some microtopography created by tree roots and vegetated hummocks. The NCWAM classification for Parker Farms was a hardwood flat. The NC NHP classification was a non-riverine wet hardwood forest.

Stone Farm: The Stone Farm site was within a private mitigation bank, which restored a non-riverine wetland (a Carolina bay), a non-riverine riparian area, and a stream, which drains from the northwest side of the bay (Figure 8). The Carolina bay and adjacent stream floodplain lies in southern Brunswick County, northeast of Calabash, NC, and adjacent to Old Georgetown Road, a paved two lane highway (Route 179). It is 4.5 kilometers north of the Intracoastal Waterway and 4 miles north of the Atlantic Ocean.

The site was located approximately 1.6 kilometers northwest from the road and at the head of a stream tributary to the Little Caw Caw Canal (a third order tributary to the Waccamaw River). The area was relatively buffered from surrounding housing and golf course development by an average of 1.6 kilometers of undeveloped land. It was 13.7 meters to 16.7 meters above sea level and within the Lumber River basin.

Re-establishment of the 145 acre Carolina bay was the first phase of this mitigation bank, which at the time of the study was still active. The entire mitigation bank encompasses 968 acres. The bay had been cleared and ditched in the 1920s. Water control structures were put on north and south side of the bay to control water by means of storage for nearby agriculture. It was also used for storing water for an adjacent golf course in more recent years. The natural historic habitat was believed to be high pocosin.

Restoration activity in the Carolina bay began in 2008, when ditches were plugged, with shallow pockets left open for semi-aquatic habitat/microtopography. The understory was selectively cleared, by removing drier, less desirable species. Wetland tree species were allowed to remain, and supplemented with further plantings of wetland trees (bald cypress, swamp tupelo, river birch [*Betula nigra*], and Atlantic white cedar [*Chamaecyparis thyoides*]). The site was closed out in 2012 after ditch plugs were reinforced and more trees were planted.

The assessment area included 0.69 acres of non-riparian wetland adjacent to the headwaters of the main stem of the restored stream and on the northwest rim of the Bay, which itself can be described as high pocosin. However, the assessment area was at the edge of this pocosin, with the soils being a mix of muck and loamy fine sand. In addition, much of the site seemed to have been permanently disturbed by re-establishment activities: an apparently scalped yellow loamy clay underlay much of the northwest portion of the site. The hydrology seemed to be driven by overbank flooding from the adjacent stream, with a typical outer Coastal Plain high water table supplying groundwater from below ground surface during high precipitation periods. The ground surface was variable, with pools, small streams, and hummocks interspersed randomly next to stands of pocosin trees and shrubs.

The assessment area of Stone Farm was a non-tidal freshwater marsh, according to NCWAM and a coastal plain semi-permanent impoundment, according to NC NHP. It was located in one of the few more open and wetter areas of the bay, with a variety of sedges. Most of the assessment area had dense shrubs (wax myrtle and blackberry [Rubus sp.]) and saplings (sweetgum) colonizing it.

Figure 8. Large-scale aerial map of Stone Farm wetland re-establishment site.

Open Canopy Reference Wetlands

Aerial photographs and on-site photographs for the open canopy reference sites are presented in Figure 9. Topographic maps for each site are located in Appendix A and additional photographs taken throughout the study are in Appendix B. All of the open canopy reference sites had perennial standing water, except for Tiger Pond, and experienced drastic seasonal changes in overall water levels.

<u>17 Frog Pond</u>: 17 Frog Pond site was located approximately 5.76 kilometers southeast of Hoffmann in northeast Scotland County, in the Sandhills Region. It was 5.76 kilometers from a paved road and at approximately the same ground elevation (121.6 meters). Ground surface at 17 Frog Pond was between 21.3 meters to 24.4 meters above typical water elevation of the nearest lake. It was located within the Sandhills Gamelands, managed by the NC WRC, within the Lumber River basin.

The assessment area was located in a shallow bowl-shaped depression, surrounded by a sandy ridge dominated by longleaf pine and loblolly pine. The delineated wetland was 4.27 acres in extent, ponded and covered with a lush herbaceous layer. The ground surface within the wetland was relatively flat with one large (several meters wide) depression towards the center. The NC NHP considers 17 Frog Pond to be a natural area of excellent representational rating, with several species of globally imperiled or critical status occurring on the site (NC NHP Data Explorer 2015).

17 Frog pond was completely open with no woody vegetation. Herbaceous vegetation varied from year to year, probably due to changes in hydrology. Grasses (*Sacciolepsis striata* and *Eleocharis melanocarpa*) dominated in 2013, while emergent (*Saggitaria isoetiformis*) were more prevalent in 2015. This site flooded most of year, but dried some in the summers.

Due to the close proximity of the Sandhills Gamelands to Camp MacKall and Fort Bragg Military Reservations, U.S. Army training in and around the study area has likely resulted in wildfires burning through the site in past decades, even when fire suppression was taking place on the rest of the landscape. In addition to unintentional fires and natural fires, this site has been managed by the NCWRC and Forest Service who have applied prescribed burns since the late-1990s, with prescription fires burning through the wetland approximately every one to three years. Fire ants were were found in the wetland basin sometime in the late 1990s, possibly affecting the local amphibian population. The last known (prescribed) fire in this wetland was in 2014, though water in the wetland prevented the fire from burning through much of the basin (WRC pers. comm. 2016).

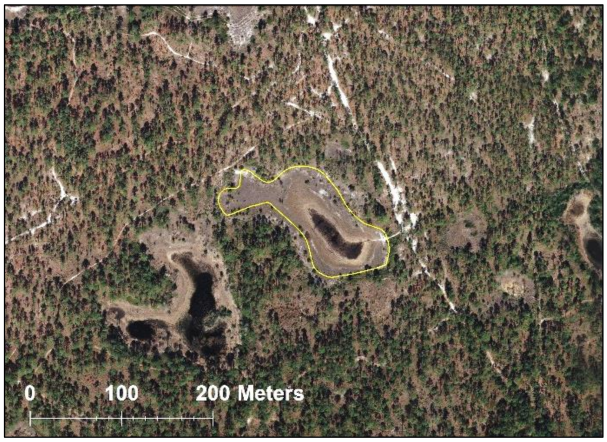
The soils in the wetland area have been mapped as Plummer and Osier sands, though the color, texture and inclusions encountered during well emplacement show significant changes from the typical pedons. A possible limiting layer was found at 56 to 66 centimeters (gray silty clay).

The NCWAM classification of 17 Frog Pond was a basin wetland and the NC NHP classification was a permanently wet depression pond.

Figure 9. Aerial photography and on-site photographs of each open canopy reference site.

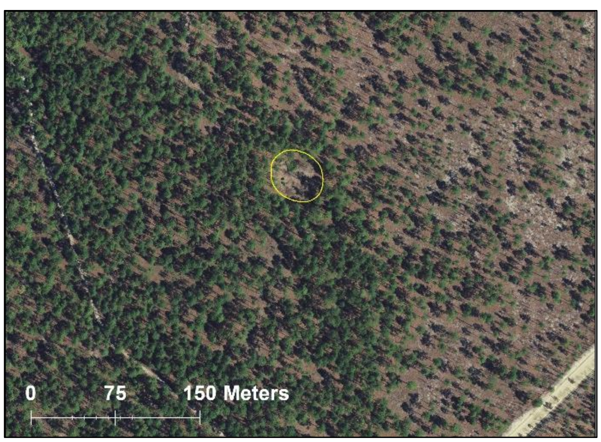
17 FROG POND ---

BRANDON'S POND --



SWAIN POND --

TIGER POND ---



Brandon's Pond: The Brandon's Pond site was located 0.354 kilometers northwest of Route 24 in Carteret County and within the Croatan National Forest, near the Atlantic Ocean in eastern North Carolina. It was 0.80 kilometers west of Broad Creek, a brackish tributary to Bogue Sound. It was only 1.6 kilometers north of Bogue Sound and approximately 6 meters above sea level. It was located in the White Oak River basin.

The upland surrounding the wetland and pond area consists of loblolly pine tree community with a scrubby oak understory growing in Kureb sand, which was gray to very light gray, almost white, in color. The upland vegetation was similar to the turkey oak scrub community of the Sandhills region. Brandon's Pond was situated within a relict bay and beach and dune ridge terrane associated with Cape Lookout that is known to be quite complex hydrologically (NC DWR Water Sciences staff, pers. comm. 2016). It was also part of a pond complex approximately two kilometers in length containing ponds of highly variable shapes and approximately same elevations as Brandon's Pond.

The area is actively managed by the NCWRC, with controlled burning and occasional wildfires occurring since the mid-1990s. Recent prescribed fires have burned through portions of the ponded area, but relatively significant duff/peat layers suggest the pond does not burn completely very often. The last prescribed burn in this area was in 2011 or 2012 (WRC pers. comm. 2016).

Similar to 17 Frog Pond, Brandon's pond was completely open with no woody vegetation. Also similarly, herbaceous vegetation was fairly dense, but varied from year to year with changes in the hydrology.

Jeff mentioned that sunfish were discovered here after one of the large storms allowed for excess flooding which affected the amphibian population.

The delineated wetland area was 2.54 acres in extent and was underlain by Murrville fine sand. There was almost no microtopography within the wetland; it was almost completely free of tree and shrub debris. Relief with surrounding upland elevations was minor. The NCWAM classification for Brandon's Pond was basin-type while the NC NHP classification was vernal pool.

Swain Pond: The Swain Pond site was located 0.37 kilometers southwest of the main entrance to the Military Ocean Terminal at Sunny Point (MOTSP) facility in Brunswick County, in far southeast North Carolina. It was immediately adjacent to the asphalted, two lane facility access road into MOTSP. The site was considered to be a limesink formation, where the topographic low that contains the wetland was formed from dissolution of underlying limey strata, most likely from the shelly upper Quarternary deposits that occur in eastern Brunswick County. There was no active management, including burning, being practiced at the site.

The site was approximately 5.9 kilometers from the lower Cape Fear River to the southeast. It seems likely to be in hydrologic connection with a nearby project site Cypress Pond, 0.55 kilometers to the west, based on measurements of changes in the pond depths. Like Cypress Pond, it was located near large relict sand mine ponds.

The surface of Swain Pond lies roughly several meters below the surface of the adjacent road bed which was 8.5 meters above sea level. Soils in the vicinity were mapped as the Kureb fine sand. The surrounding upland area was dominated by loblolly pines and scrub oak, reminiscent of the turkey oak scrub of the Sandhills region to the west.

Historic drought had allowed for upland loblolly pine to establish along the north and south sides of Swain Pond. By 2015, a portion of the pines had died off but was still standing. The site was dominated by a dense carpet of submerged aquatics (*Zanichella palustris*, *Juncus repens*) and sphagnum moss. Scattered sedges and herbs were located along the edge.

The 0.38 acres of wetland was contained within a shallow concave topographic low on the north side of the MOTSP access road. Relief between the upland and wetland and/or pond surface elevation was marked (2-3 meters over a distance of 20 or so meters). There was not much microtopography within the wetland area except what was provided by several large logs and downed limbs. The NCWAM designation for Swain Pond was basin wetland and the NC NHP classification was permanently wet depression pond.

Tiger Pond: The Tiger Pond site was located 0.32 kilometers northwest of Gum Swamp Road, a dirt track within the Sandhills Gamelands, adjacent to Hoffmann, NC in Scotland County. It was within the Lumber River Basin system and the Sandhills physiographic area. The pond was at 106 meters elevation, 30 meters higher than the two closest water bodies, Gum Swamp Lake and Crawford Lake, which lie approximately 1.6 kilometers to the northeast and southeast, respectively. There was little relief in the overall site vicinity.

The assessment area was surrounded by dry longleaf pineland (Sorrie 2011), with dominant vegetation including mature longleaf pine trees and clumps of turkey oak shrubs and grass as understory. The wetland area was quite small, only 0.39 acres and included an open canopy vegetation community of scattered shrubs and diverse herbaceous species. Unlike the other open canopy reference sites, this site typically did not have standing water, or only in the deep hole in the center. The ground surface has an overall concave bowl configuration, with one markedly deep (~ 1.5 meter) depression towards the center. There was little microtopography within the wetland, other than that provided by shrub and grasses stems.

This site has experienced prescribed burning since the late 1990s, with recently set fires burning through the wetland about every two to four years. The open canopy suggests that this site has burned frequently even before prescribed burning became commonplace on the Sandhills Gamelands. The last known prescribed fire in this wetland was in 2013 (WRC pers. comm. 2016).

The geologic location of Tiger pond was in the sand deposits of the Pinehurst Formation. Both upland and wetland areas were underlain by the Ailey loamy sand, with the wetland soil exhibiting hydric modifications. No limiting layer was encountered at depths in excess of 76 cm during the emplacement of the monitoring well. The site was a NCWAM basin wetland and a NC NHP vernal pool.

Enhancement Wetlands

Aerial photographs and on-site photographs for the enhancement sites are presented in Figure 10. Topographic maps for each site are located in Appendix A and additional photographs taken throughout the study are in Appendix B.

Block T Pond: Block T Pond was located 1.11 kilometers due east of Rockingham Speedway motor race track in Hoffmann, Richmond County, within the Sandhills Gameland managed by the NC WRC. Located within the Lumber River basin, it was 0.45 kilometers from paved road and railroad tracks. Ground surface at Block T Pond was almost 27.4 meters above typical water elevation of the nearest waterbody. It was geographically and geologically located within the Sandhills region.

Historic photos from the late 1940s show that this site was open canopy, likely because of natural wildfires, though the site developed a closed canopy of pines and hardwoods by the mid- 1980s. In 2011, all canopy trees were removed from the site by the NC WRC using heavy equipment, and the site was intentionally burned in 2011 and 2013 (WRC pers. comm. 2015).

The surrounding upland was a combination of dry longleaf pineland and turkey oak scrub. The upland pine trees were relatively mature with mainly just an herbaceous layer underneath their canopy. Relief was marked for the area, with at least 4.57 meters of elevation difference between the surrounding hills and the elevation of the wetland proper.

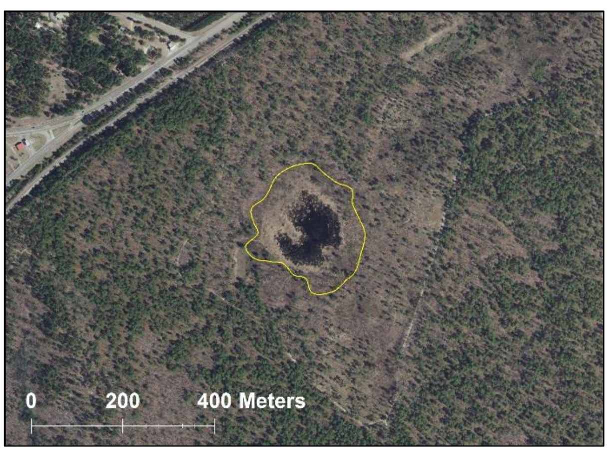
The wetland area was roughly circular and, at 12 acres was quite large for a typical "isolated" wetland in North Carolina. The hydrology seemed to be heavily dependent on runoff from the surrounding uplands. A shallow pool was located within the middle of the wetland area. Ground surface was typically flat, with several large (up to 2.5 meters high) mounds of timber left after the enhancement activities.

Block T had a diverse herbaceous layer that was more prevalent in the latter two years of the study when the site was less inundated. This site seasonally flooded, but it usually had water in the deepest sections Water levels varied from four feet deep in 2013 to less than a foot during the driest season of 2015.

Soils in the wetland area indicate either a modified Pelion (upland) component or a Bibb component, medium to coarse sandy material which includes a clay layer that seems to be the ponding mechanism for the vernal pool. The NCWAM classification for the site was basin type. The NC NHP classification was vernal pool.

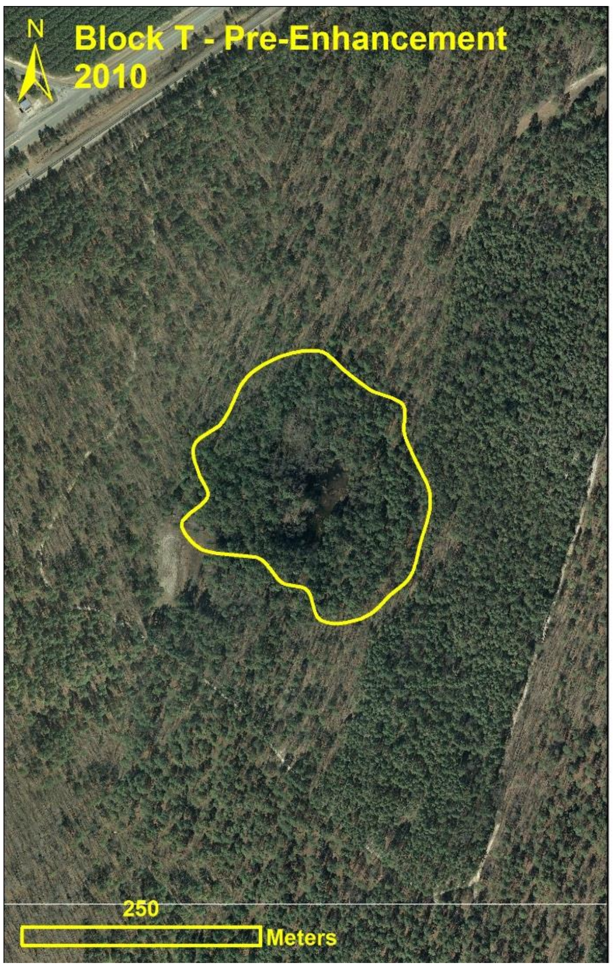
Figure 10. Aerial photography and on-site photographs for each enhancement site.

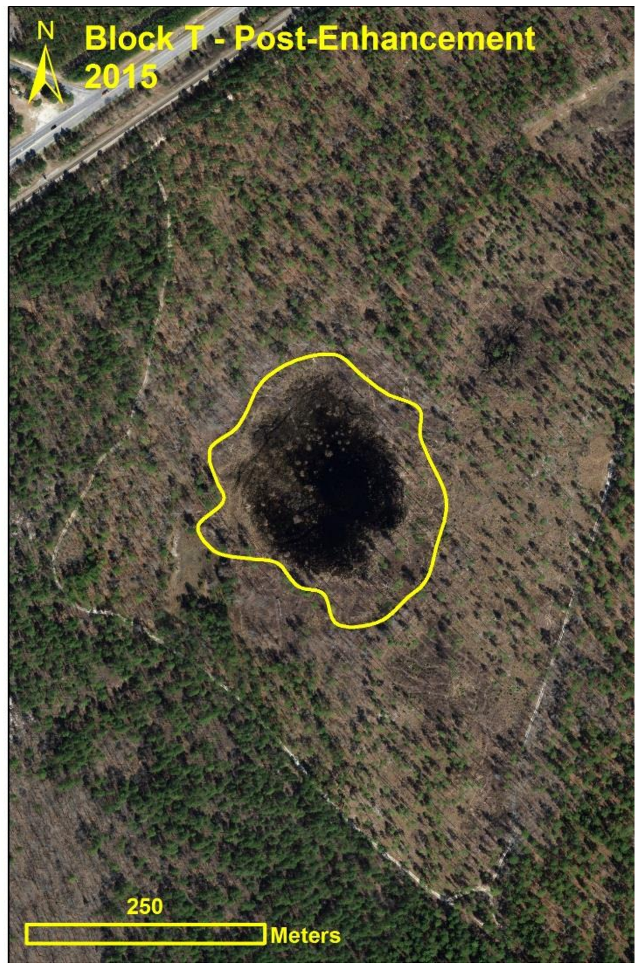
BLOCK T --



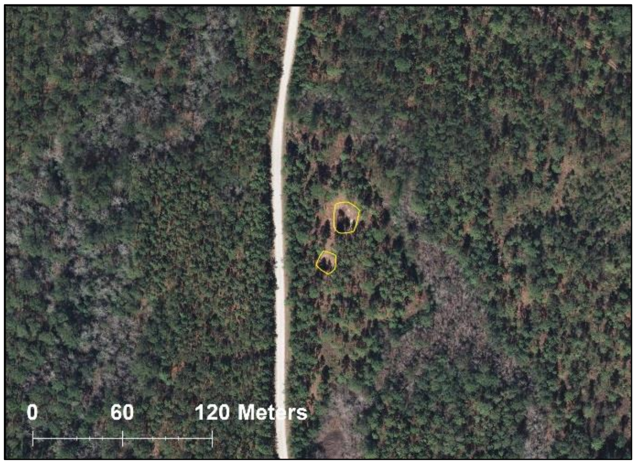
BLOCK T PRE-ENHANCEMENT (2010)

BLOCK T POST-ENHANCEMENT (2015)





BRASWELL PONDS ---

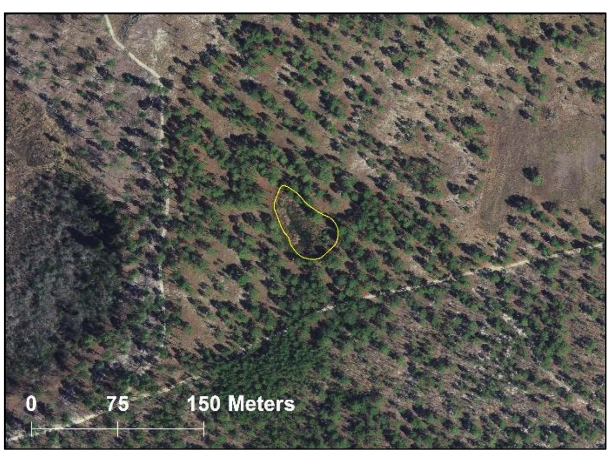


BRASWELL POND (SOUTH) PRE-ENHANCEMENT (2010)

BRASWELL POND (SOUTH) POST-ENHANCEMENT (2015)

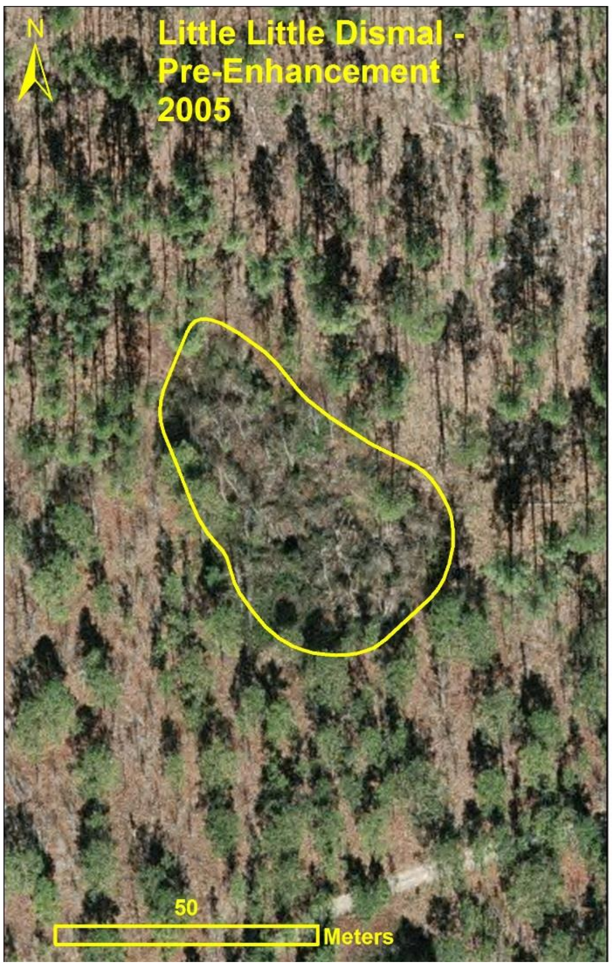
*2015 aerials were unavailable for this area

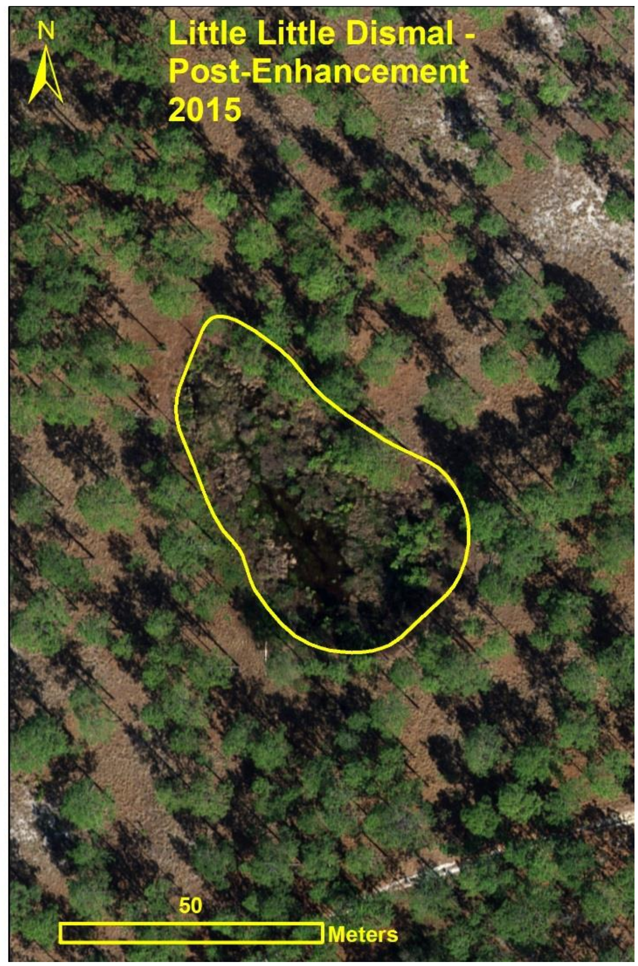
LITTLE LITTLE DISMAL ---



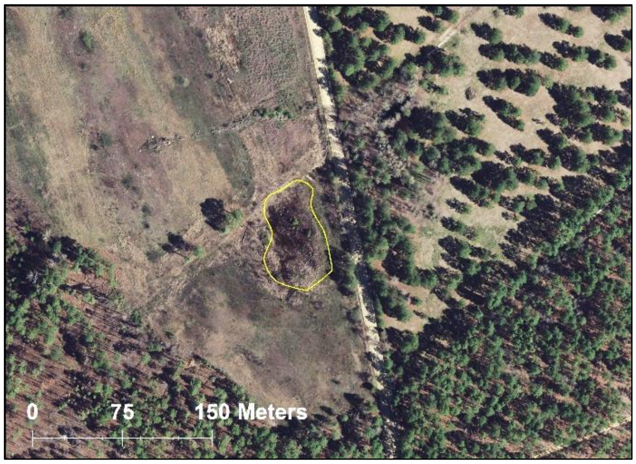
LITTLE LITTLE DISMAL PRE-ENHANCEMENT (2009)

LITTLE LITTLE DISMAL POST-ENHANCEMENT (2015)



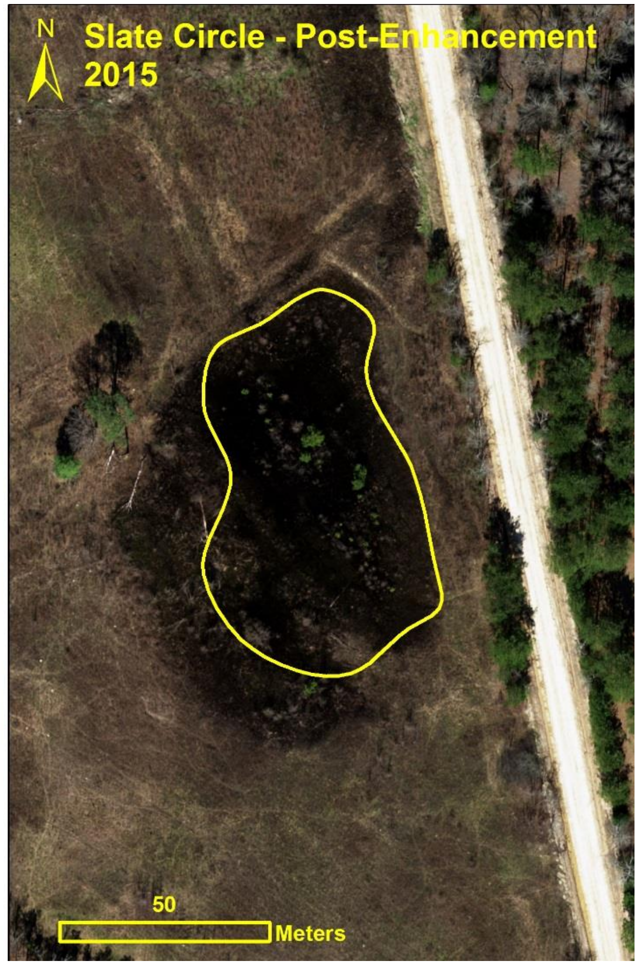


SLATE CIRCLE ---



SLATE CIRCLE PRE-ENHANCEMENT (2009)

SLATE CIRCLE POST-ENHANCEMENT (2015)



Braswell Ponds: The Braswell Ponds site was located 3.15 kilometers east of NC Highway 58 and adjacent to Forest Road 3014 in the Pocosin Wilderness of the Croatan National Forest in Carteret County. The forest road was approximately 6 meters wide and consists of unpaved gravel and sand. The surface of the wetland and ponds were at or slightly below road surface elevation (9.4 meters AMSL). The site was located in the White Oak River basin.

The site was located in a limesink geomorphic environment. Two small, perennial ponds were separated by less than 10 meters of upland and were aligned north to south, the larger and deeper pond to the north. The depressions in which the ponds have formed have been presumably created by dissolution of calcareous strata somewhere below ground surface. A thin strip of wetland vegetation surrounded both of the open ponds, with the entire wetland area totaling only 0.098 acres.

Historically the site was relatively open canopy (to the early 1980s), but had become overgrown with shrubs and trees by the 2000s because of lack of natural fire episodes and possibly drought. In 2011, an enhancement effort was initiated, with all trees and shrubs removed using chainsaws and other hand equipment. The site has not been intentionally burned in recent history (at least 10 years) (WRC pers. comm. 2015).

The north pond ground surface was bowl-like, steeply sided with stumps and logs still present on the bottom from the previous enhancement activities. A dense wall of red maple and sweet gum saplings and shrubs (titi) on the rim of this pond separates the open water from adjacent upland. The south pond has a shallower and flatter bottom ground surface. The saplings and shrubs immediately surrounding this pond were not as dense as they were around the north pond.

The northern deeper pond was perennially flooded (up to 3 feet) and vegetated with various sedge species. The southern pond was seasonally flooded and only vegetated with switch cane on the edges along with red maple and sweetgum.

A complex of upland and hydric soils was found in the area, with Baymeade sand, Leon sand and Masontown mucky loam interspersing with Onslow loamy fine sand units. The upland surrounding both ponds was covered with typical coastal plain loblolly pine flat vegetation of mainly mature pine trees underlain by shrub and herbaceous understory layers. The immediate upland surface was up to 3.04 meters higher in elevation from the wetland/pond elevations.

The NCWAM schema classifies this site as comprising two basin wetlands. The NC NHP type(s) was (were) small depression ponds.

Little Little Dismal Pond: The Little Little Dismal Pond site was located 0.63 kilometers northeast of Marks Creek Church Road, a two-lane dirt track within the Sandhills Gameland, adjacent to Hoffmann, in Richmond County. It was only 1.12 kilometers northwest of another project site (Tiger Pond) and was found at 121 meters above sea level. This was 15 to 18 meters above the elevation of the nearest body of water, 0.8 kilometers to the northeast. It was 45 meters above the nearest named body of water (Gum Swamp Lake), 2.95 kilometers to the east. It was geographically located within the Sandhills region and the Lumber River basin.

Previous to this study, the site contained a dense shrub layer with numerous canopy trees, suggesting it had not been burned naturally with any frequency in many decades. In 2010, an enhancement effort was applied to the wetland with all canopy trees and most of the shrub layer removed with heavy equipment and/or by hand. The site has since undergone prescription burning twice, in 2011 and 2013 (WRC pers. comm. 2015).

Surrounding upland was covered by a pine flatwood vegetation community with mature loblolly and longleaf pine dominating over mostly grassy herbaceous and (sparse) shrub layers. There was almost no relief between the adjacent upland ground surfaces and the surfaces within the wetland. There was another larger bay-like wetland 0.16 kilometers to the west and at slightly lower elevation.

The wetland comprises 0.50 acres. It was generally flat with some large piles of logs left over from the enhancement.   A dense shrub and sapling layer surrounds a ponded oval area which contains a lush herbaceous layer. Some of the grass clumps produce microtopographic hummocks.

The surrounding soil was mapped as a Wakulla/Candor upland unit. However, the wetland area was also underlain by a circular and/or ringed assemblage of Paxville fine sandy loam surrounding an inclusion of Johnston mucky loam (ponded area). A true limiting or water perching layer was not reached during the emplacement of the well (to >211 centimeters).

The site was considered by the NC NHP to be a vernal pool. Little Little Dismal Pond was an example of an NCWAM basin wetland.

Slate Circle Pond: Slate Circle Pond was located 6.08 kilometers west of Drowning Creek, the upstream main stem to the Lumber River and 9.4 kilometers east of Hoffman, in Scotland County. It was 1.54 kilometers west of the nearest paved road and at 106 meters of elevation, 18.3 meters above the water surface elevation of the nearest named water body (Big Muddy Lake). It was located within the Sandhills Gameland managed by the NC WRC.

Historical aerial photography shows the site to have been a seasonally ponded location surrounded to the north, west and south by active farm or pasture land. The adjacent farmland was fallow at the time of the study. To the east the site was bordered by a two-lane dirt road, a mixture of longleaf pines and open range-land. The road surface was at some 1.5 meters higher in elevation than the wetland ground surface.

An enhancement effort at the site was begun in 2010; canopy trees were removed using heavy equipment and a ditch was removed and/or plugged. Prescribed burning took place in 2010 and again in 2012 (WRC pers. comm. 2015).

The wetland area was 0.89 acres in extent. Dense shrubbery surrounds it and was interspersed with herbaceous vegetation. There were no longer any mature trees within the wetland boundary. The ground surface was relatively flat and still retains some relict large logs and stumps left from enhancement work.

The site was located in mapped Pelion loamy sand, between units of hydric Rains loamy sand. The wetland area consists of either an inclusion of Rains loamy sand or an extension of a nearby Rains unit. A (presumably) hydrologic limiting layer of extremely tight silty clay was encountered at a depth of 76 centimeters at the well site during emplacement. Historical air photographic evidence indicated this layer may extend 250 meters to the north and east. The NCWAM system classifies Slate Circle Pond as a basin wetland and NC NHP classifies it as a vernal pool.

LANDSCAPE SETTING AND RAPID ASSESSMENTS

All sites in this study were assessed using the Landscape Development Intensity Index (LDI) (Brown and Vivas 2005) which estimates the potential impacts from anthropomorphic influences on land cover by evaluating land cover in a designated area. LDI values are essentially human-related disturbance scores that are associated with intensity of the land-use based on non-renewable energy flow.

Amphibians that are dependent on wetlands for reproduction often spend most of their lives in habitats away from wetlands, and return to wetlands to breed. Additionally, once larvae metamorphose, they can disperse long distances away from wetlands. To determine the condition of the land and habitats that wetland-dependent amphibians could encounter during dispersal, a literature search was first undertaken to discover maximum dispersal distances of the potential amphibian species of Coastal Plain wetlands (Table 2). Long dispersal distances of 2 to 5 kilometers (1.2 to 3.1 miles) from natal areas, even within a matter of weeks, have been reported for amphibians (Dole, 1971; Pough et al.1998; Petranka 1998; Sinsch 1997), and the average maximum dispersal distance found for potential Coastal Plain wetland amphibians was 1,271 meters (0.8 miles). Therefore, a conservative buffer distance of 1 mile was chosen for the LDI analysis. The LDI was also calculated for a 500-meter buffer around each wetland site (or study area, for re-establishment sites) to assess the area immediately surrounding the wetland sites.

The LDI was calculated by delineating land uses/covers using recent aerial photographs and calculating the percent of the total spatial extent occupied by each land use/cover. Each land use or land cover was assigned a coefficient (Table 3), which was multiplied by the aerial extent of that land use/cover. The following equation from Brown and Vivas (2005) was used to determine the LDI value for each wetland 500-meter buffer and extended buffer (1 mile).

$$\text{LDI} = \sum(\%\text{Lu}_i * \text{LDI}_i)$$

$\%\text{Lu}_i$ = percent of the total area of influence with land use i
LDI_i = LDI coefficient for land use i

Table 2. Maximum dispersal distances for potential Coastal Plain wetland-dependent amphibians. Some species had no reported dispersal distances.

Common Name	Scientific Name	Detected in This Study	Max Distance (m)	Source
Southern Cricket Frog	*Acris gryllus*	✓	562	Micancin, J.P. 2010.
Mabee's Salamander	*Ambystoma mabeei*	✓	800	Hardy, J.D. 1969.
Eastern Tiger Salamander	*Ambystoma tigrinum*	✓	600	Smith, M.A., and D.M. Green. 2005.
Oak Toad	*Bufo quercicus*	✓	80	Dodd, C.K. 1994.
Southern Toad	*Bufo terrestris*	✓	1609	Smith, M.A., and D.M. Green. 2005.
Chamberlain's Dwarf Salamander	*Eurycea chamberlaini*			
Eastern Narrowmouth Toad	*Gastrophryne carolinensis*	✓	914	Dodd, C.K. 1996.
Pine Barrens Treefrog	*Hyla andersonii*	✓	106	Semlitsch, R.D., and J. R. Bodie. 2003.
Cope's Gray Treefrog	*Hyla chrysoscelis*	✓	1100	Roble, S.M. 1979.
Green Treefrog	*Hyla cinerea*	✓	200	Piacenza, T. 2008.
Pine Woods Treefrog	*Hyla femoralis*	✓	190	Piacenza, T. 2008.
Barking Treefrog	*Hyla gratiosa*	✓		
Squirrel Treefrog	*Hyla squirella*	✓	800	Piacenza, T. 2008.
Eastern Newt	*Notophthalamus viridescens*	✓	1000	Smith, M.A., and D.M. Green. 2005.
Spring Peeper	*Pseudacris crucifer*	✓	300	Davis, S.L. 1999.
Southern Chorus Frog	*Pseudacris nigrita*			
Little Grass Frog	*Pseudacris ocularis*	✓		
Ornate Chorus Frog	*Pseudacris ornata*	✓	55	Semlitsch, R.D., and J. R. Bodie. 2003.
Mud Salamander	*Pseudotriton montanus*			
Carolina Gopher Frog	*Rana capito*	✓	3400	Hall, J. 2015.
American Bullfrog	*Rana catesbeiana*	✓	1600	Smith, M.A., and D.M. Green. 2005.
Green Frog	*Rana clamitans*	✓	4800	Smith, M.A., and D.M. Green. 2005.
Southern Leopard Frog	*Rana sphenocephala*	✓	5200	Dole, J.W. 1971.
Carpenter Frog	*Rana virgatipes*	✓		
Eastern Spadefoot	*Scaphiopus holbrookii*	✓	825	Smith, M.A., and D.M. Green. 2005.
Many-lined Salamander	*Stereochilus marginatus*			

Table 3. LDI Coefficients used for various land cover/land use descriptions (adapted from Brown and Vivas, 2005).

Land Cover/Land Use Description	LDI Coefficient
Natural Area - land and water, wetlands	1
Natural Area with modifications, ie. ditches, ATV tracks, etc.	1.5
Unmanaged Herbaceous - woodland pastures, feed lots	2
Immature mitigation wetland	2
Shrubby areas recovering from logging	2
Managed Herbaceous - pasture, regularly mowed areas	3
Pine Plantations	3
Logged - recently logged areas, clearcuts	3
Impounded water	4
Barren - dirt roads, land cleared for construction	4.4
Agriculture - row crops	5
Agriculture - poultry/swine houses and lagoons	7
Low Density Development - low to medium density residential, high intensity recreational/open space, 2 lane highways	7
High Density Development - high density residential, low intensity commercial, 4 lane highways	8
Industrial	8.5
Mining	10

The North Carolina Wetland Assessment Method (NCWAM) was also used to assess each wetland site. This is a rapid assessment method that results in a rating (High/Medium/Low) for various wetland functions (hydrology, water quality, and habitat) (NC Division of Water Resources 2008). The Ohio Rapid Assessment Method v.5.0 (ORAM) was another rapid assessment method used to gauge habitat quality of each of the wetland sites (Ohio Environmental Protection Agency 2001). ORAM contains six rapid assessment metrics which produce a numeric score for the habitat quality of a wetland: 1) wetland area, 2) upland buffers and surrounding land use, 3) hydrology, 4) habitat alteration and development, 5) special wetlands, and 6) plant communities, interspersion, and microtopography. Metric 5, which is specific to Ohio wetlands, was not used in the assessment. The maximum score for a high quality wetland was 90 without the use of metric 5.

HYDROLOGY MONITORING AND PRECIPITATION DATA

Monitoring wells (2” diameter PVC) were installed in the approximate center of the wetland study sites so hydrological variations could be observed. Methodology outlined by the US Army Corps of Engineers was followed in installing the monitoring wells (Sprecher 2000). Wells were installed vertically two feet below the surface, except in cases where there was standing water and wells could not be installed below ground surface. A solid plug was screwed into the end of the screen before it was placed into the open borehole. A sufficient length of solid PVC riser was assembled to permit the PVC well to extend approximately two to five feet above the land surface. The wells had 0.01 inch slats along the lower 18 inches for water flow. Clean filter sand was poured into the annular space between the borehole and the PVC well until the resulting sand filter pack extended above the top of the well screen slots. A bentonite seal was placed in the remaining annular space above the sand filter pack. An expanding plug was placed at the top of the PVC to prevent foreign matter from entering the well. A small hole approximately 1/8-inch diameter was drilled in the PVC casing approximately four inches below the top to allow pressures inside the well to equilibrate. This allowed for water level fluctuations in the well to be unhindered by pressures inside the sealed well. Wells were installed for at least 24 hours before the first water level readings were recorded.

Vented 500 Level In-Situ transducers were installed in each monitoring well and set to read every hour. A measuring point was marked on the top of the well casing. Water level depth was measured by hand each time the well data were downloaded and compared to the most recent hourly reading to ensure accuracy of the transducer. Transducers with >0.1 ft inaccuracies were replaced. Well data was downloaded to a laptop computer every three months using In-situ software Version 5.

Precipitation data were also obtained to examine the relationship between water levels and precipitation in the study wetlands. The data were obtained by searching maps on the website of the National Ocean and Atmospheric Administration (NOAA) National Centers for Environmental Information to locate and download the nearest precipitation station data for each wetland. Daily summaries were used in graphing and analysis (NOAA 2016).

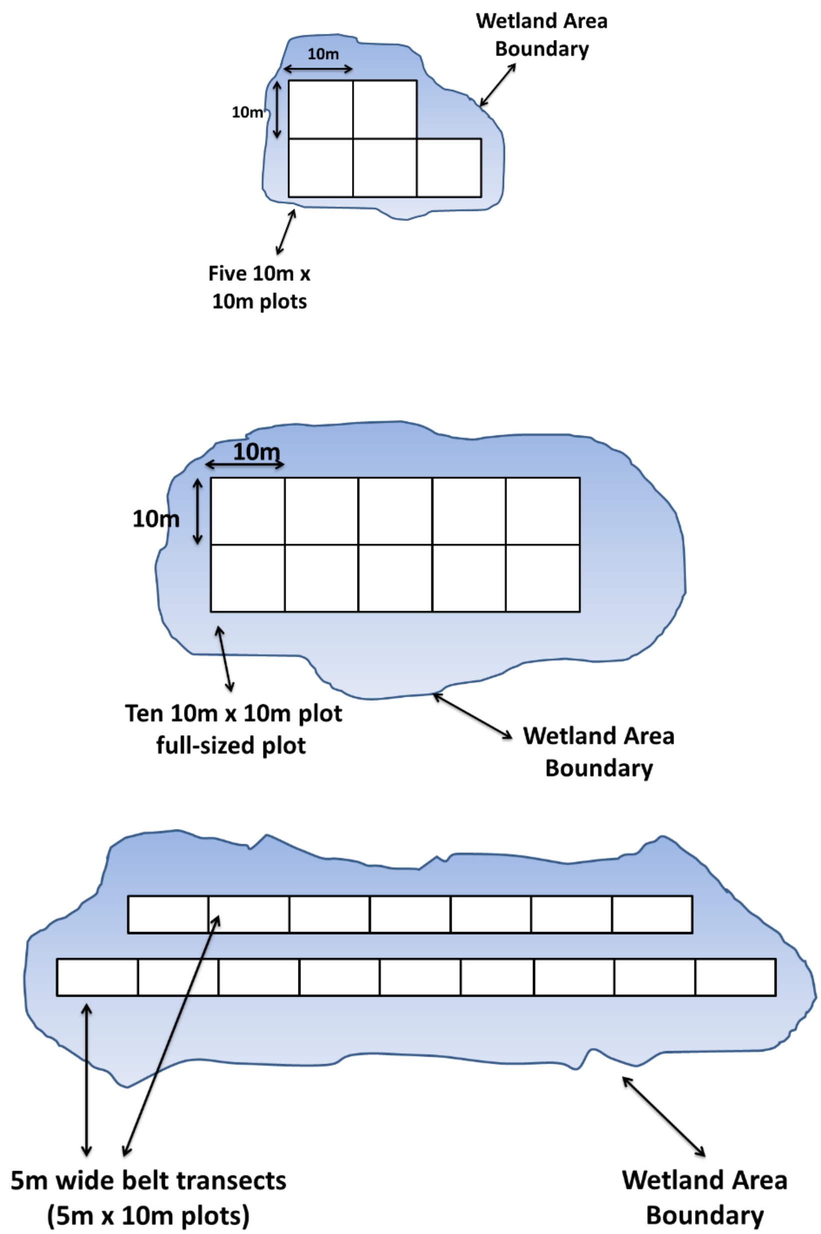
VEGETATION SAMPLING

The wetland survey site area and surrounding buffer were surveyed during the first and third growing season after the survey sites were delineated. Vegetation vertical strata structure, growth form, and ground surface attributes along with dominant and invasive species were recorded at each wetland site. The vegetation vertical strata structure and woody debris coverage were recorded for the surrounding buffer. The pool size, pool depth and vegetation coverage were also surveyed quarterly during the hydrology monitoring. The National Wetland Condition Assessment (NWCA) vegetation survey methods (US EPA 2011) and the North Carolina Vegetation Survey Protocol (CVS) (Peet et al. 1998) were used as templates for developing the vegetation sampling methodology for this study. The vegetation sampling design and survey methodology are described in the following sections. Vegetation and pool depth field forms are in Appendix C.

Vegetation Sampling Design

The vegetation sampling design was flexible in nature for usability at study sites which ranged in shape and size. The delineated boundary shape of the wetlands dictated how the survey design was set up. A series of 10 m wide belt transects divided into increments of 10 m (i.e. adjacent 10 x 10 m^2 plots) were used for all sites except one, where 5m x 10m plots were used in some cases due to the shape of the wetland. We aimed to survey 1000 m^2 of each wetland, unless wetlands were too small to accommodate 10 full 100 m^2 plots, in which case, as many plots as possible were placed to sample the entire wetland (Figure 11). For large sites ($\geq$0.50 Ac), belt transects that bisected the center of the widest section of the wetlands were used. Additional belt transects were added, as needed, to ensure a minimum of 1,000 m^2 was surveyed. Vegetation vertical structure, growth form, and ground surface attributes were estimated for each plot. Plot corners or every 10m along the center line of the belt transects were flagged and the ends and center of the belt transects were recorded with GPS. The sampling design was also sketched on data collection forms.

Figure 11. Example vegetation plot survey designs.



Coverage

Coverage was defined as “the percentage of ground surface obscured by the vertical projection all above ground parts of a given species onto that surface”. Individual species that were either rooted in or overhanging the survey plot were included in the cover estimates. Percent cover was estimated using the midpoint of each cover class.

- Trace (1–2 individuals only, ≤ 0.25m^2)
- Trace - 1 m^2
- 2 m^2(1m x 2m)

- 2-5 m^2 (1m x 2m to 1m x 5m)
- 5–10 m^2 (1m x 5m to 1m x 10m)
- 10-25 m^2 ($5m^2$)
- 25–50 m^2 ($5m^2$ to 5m x10m)
- 50–75 m^2 (5m x10m to $8.7m^2$)
- 75–95 m^2 ($8.7m^2$ to 9.7 m^2)
- 95–100 m^2 (9.7 m^2 to 10 m^2)

Vegetation Vertical Strata Data

Vertical strata coverage was recorded for vascular species for the following height classes.

- 0-0.5m (herbaceous plants, low shrubs, and tree seedlings)
- >0.5-1m (herbaceous plants, low shrubs, and tree seedlings)
- >1-2m (tall herbaceous plants, medium sized shrubs, and tree saplings)
- >2-5m (very tall herbaceous plants, tall shrubs, and tree saplings and short trees)
- >5-15m (very tall shrubs, short to mid-sized trees)
- >15-30m (tall trees)
- >30m (very tall trees)

Vegetation by Growth Form Coverage

Overall coverage by growth form was estimated for the following non-vascular and vascular groups:

- Filamentous or matt forming algae
- Ground lichens (total cover of lichens on the ground substrate and fallen logs or roots)
- Bryophytes (mat-forming mosses, sphagnums, and thalloid liverworts)
- Submerged aquatic vegetation (SAV) (Aquatic vegetation that would be found under water during normal conditions)
- Floating aquatic vegetation (Vascular species not rooted in sediment, floating on the water surface)
- Emergent vascular vegetation (Vascular species that under normal conditions emerge above the water surface when standing water exists)
- Shrubs
- Broadleaf small trees and large trees (e.g. *Ilex opaca* and *Fraxinus pennsylvatica*)
- Lianas, vines, and epiphytes
- Coniferous trees (e.g. *Juniperus* spp., *Pinus* spp., *Taxodium* spp.)

Ground Surface Attributes and Woody Debris/Snags

Ground surface attribute coverages were estimated using defined cover classes and mean percent cover (across plots) was calculated for each of the following:

- Standing water with no vegetation (B)
- Standing water with SAVs, moss, floating aquatics or algae (C)
- Standing water with herbaceous emergents (D)
- Standing water with woody emergents (E)
- All standing water = B+C+D+E (from above)
- Exposed soil/sediment

- Broadleaf litter (attached or detached dead herbaceous matter or broadleaf tree litter). Litter for this study was defined as attached or detached dead herbaceous matter that has retained its shape and has not decomposed.
- Coniferous litter. Litter for this study was defined as attached or detached dead herbaceous matter that has retained its shape and has not decomposed.
- All litter (broadleaf + coniferous)
- Dead woody debris <5 cm dbh
- Dead woody debris >5 cm dbh
- Standing dead shrubs and saplings <5 cm dbh
- Standing dead shrubs and saplings >5 cm dbh
- Standing dead snags >5 cm dbh
- Gravel 2mm to 25mm.*
- Rocks >25mm.*

* Cover of gravel and rocks were dropped from analysis because they were nearly non-existent in the wetlands of this study. Substrates in the wetlands of this study were primarily sandy or mucky.

Dominant Vegetation Species Coverage

The dominant vegetation coverage and invasive plant species present were also recorded (Figure 12). Four growth forms; herbaceous, shrub, tree (including small trees), and lianas/vines/epiphytes, for the dominant vegetation were identified and estimated for coverage separately. Herbaceous species that covered ≥5% of a site and woody shrubs, trees, lianas/vines/ephiphytes that covered ≥10% of a site were identified and estimated by cover class. Exotic invasive plant species that were observed during the survey and not considered to be a dominant species were also recorded as present. Unknown vouchers were collected, pressed, and labeled with a site collection number. Vouchers were identified using Radford et al. (1968), USDA Plants Database (USDA, NRCS 2015), and Weakley (2012).

Surrounding Upland Buffer Survey

In the surrounding upland buffer, five to ten 10m^2 plots were surveyed using the defined coverage classes for the following growth forms (see the “Vegetation by Growth Form Coverage” section above) and woody debris:

- Herbaceous vascular vegetation and matt forming mosses
- Shrubs
- Broad leaf small trees and large trees
- Lianas, vines, and epiphytes
- Coniferous trees
- Detached woody debris ≤5 cm diameter located on the ground
- Detached woody debris >5cm diameter

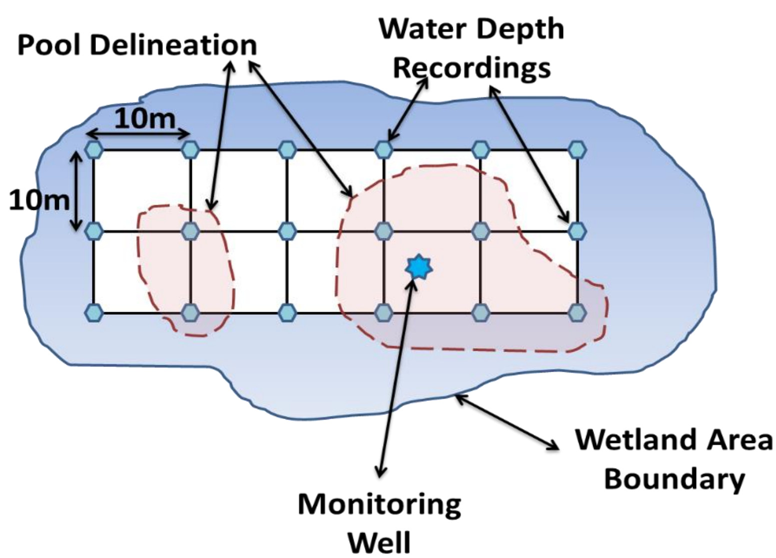
Pool Size andWater Depth

During each hydrology monitoring event, the pool size and water depth were recorded. The pool size was delineated and recorded using GPS. Water depth was recorded at plot corners or every 10 meters along the center-line of the 5m wide belt transects (Figure 13).

Growing season dates were obtained for each site from the Natural Resources Conservation Service (NRCS 2016).

Figure 12. Photograph of scientists recording vegetation data in an enhancement wetland (Braswell Pond).

Figure 13. Pool delineation and water depths recording locations example.



Pool Volume Calculations

Water depth measurements and pool boundaries were input into ArcGIS ArcScene software (ESRI 2014) to create a three-dimensional TIN (triangulated irregular network) surface of the bottom of the pool, from which volume was calculated using the Polygon Volume tool. When multiple pools were present on a site, volumes were added together to obtain a total volume.

MACROINVERTEBRATE SAMPLING

The macroinvertebrate sampling method was developed after consulting EPA wetland assessment methods; wetland assessment methods in Maine, Ohio, Florida and Minnesota; early NC DWR wetland assessments (Baker et al. 2008); and NC swamp stream methods. This method was designed to collect quantitative and semi-quantitative samples. Aquatic macroinvertebrates were collected twice from each site. Sampling occurred once per year during the first two years, in the early spring, when wetlands were at, or just past, their maximum water level.

Quantitative Sampling

Quantitative sampling was done with a bottomless sieve bucket (Figure 14). This method was described by Ken Fritz (EPA) for collecting macroinvertebrates in headwater streams (Fritz et al. 2006). One or two sieve bucket samples were collected at randomly determined locations within different habitats (when possible) in each wetland in water depths <0.4m. The open-bottom bucket was pressed into the substrate until resistance was met, and 5-8 cm of substrate collected and sorted for macroinvertebrate collection. The samples were elutriated and preserved separately in the field. Samples were later picked in the lab and identified to the lowest possible taxonomic level.

Figure 14. Photograph of bottomless sieve bucket used in macroinvertebrate sampling.

Semi-Quantitative Sampling

Semi-quantitative sampling was done using a D-frame net and a fine-mesh sampler (called a chironomid "getter") for sampling smaller invertebrates (especially Chironomids), and were based roughly on NC DWR swamp sampling methods (NC DWQ 2012). The chironomid "getter" was used because it can obtain taxa found in situations where there is a high amount of organic matter. When habitat was available, two "getters" were collected by washing hard substrates found within the wetland (mostly logs). A sweep was performed by standing in one place, and sweeping an area of approximately two meters by two meters using a D-frame net multiple times until the area was completely sampled (Figure 15). One to four separate sweeps were collected at each wetland wherever possible – at least one sweep was collected in each major habitat found in the wetlands (shallow water [0.5m or less], deep water [0.75 meter and deeper], and vegetation [terrestrial and aquatic vegetation in standing water]) (Figure 16). Samples were kept separate and returned to the lab for picking and identification to the lowest possible taxonomic level in the lab.

The "Aquatic Insects and Oligochaetes of North and South Carolina" by Brigham et al. (1982), "The Water Beetles of Florida" by Epler (2010), "Identification Manual for the Larval Chironomidae (Diptera) of North and South Carolina" by Epler (2001), and "The freshwater amphipod crustacean (Gammaridae) of North America" by Holsinger (1976) along with other taxonomic keys were used to identify aquatic macroinvertebrate taxa.

Figure 15. Examples of a D-frame sweep net and fine-mesh sampler for sampling invertebrates.

Photos: benmeadows.com and NCDOT macroinvertebrate survey protocols (2009).

Figure 16. Photograph of scientists sampling for macroinvertebrates using sweep net.

AMPHIBIAN SAMPLING

This study combines results over three years after the manner of Pechmann et al. (2001) and others (Gonzalez 2004) to reduce the impact of natural variation on species detection. Amphibians were sampled in each wetland using a variety of methods, including auditory sampling, dipnet sampling, and general opportunistic surveys (US EPA 2002a). Sampling was conducted late winter through July of each year. These methods were designed to determine presence of species using each wetland site, but not necessarily abundance.

Auditory Sampling

The auditory sampling was conducted by placing automated acoustic recorders (Songmeter SM2 dataloggers, Wildlife Acoustics, Inc.) at each site (Figure 17). These dataloggers record audio of calling male frogs and toads and were analyzed by manual listening to recordings. Dataloggers were programmed to record for five minutes at the top of every hour from just after sunset for seven hours (35 recording minutes/night). Loggers were deployed at all 16 sites winter, spring, and summer if/when water was present in each wetland. Data were downloaded and analyzed for each wetland, yielding anuran species presence information for each year and site. Recording dates for each of the three years by site can be found in Table 4. During the course of the study, equipment difficulties occurred on some sites, including recorder malfunctions, one theft, and one burn. However, data were obtained on all sites for at least two years, and most often all three years.

Table 4. Dates of froglogger recording for each site in all years of the study.

Site	Recording Schedules			Notes		
	2013	2014	2015	**2013**	**2014**	**2015**
17FrogPond	6/7-7/12	1/13-7/16	3/5-4/11	dry until 6/7	wet all year	recorder malfunction after 4/11
Block O	6/18-7/12	1/13-7/16	3/5-7/23	dry until 6/18	dry 6/1	dry during most of year
Block T	4/11-7/12	1/13-7/16	3/5-7/23		wet most of year	dry by 7/25
Brandon's Pond	4/11-7/12	1/13-7/16	3/5-7/1	dry by 7/17		recorder malfunction after 7/1
Braswell Ponds	4/11-7/12	1/13-7/16	3/5-5/9		wet all year	recorder malfunction after 5/9; wet all year
Cypress Pond	4/11-7/12	1/13-7/16	3/5-7/23		wet all year	wet all year
Dover	4/11-7/12	1/13-5/26	N/A		recorder malfunction after 5/26	recorder data corrupted (ants)
Gum Pond	4/11-6/15	1/13-7/16	3/5-7/23	recorder malfunction after 6/15	dry 5/20	dry by 7/5
Juniper Bay	4/11-7/12	1/13-7/16	3/8-7/23			
Little Little Dismal	4/11-7/12	4/17-7/16	3/6-7/23		recorder malfunction until 4/17; dry by 7/1	dry by 5/15
Parker Farms	4/11-7/12	1/13-7/16	N/A			recorder stolen
Pulpwood Pond	6/7-7/12	1/13-7/16	3/6-7/23	dry until 6/7	dry 7/1	dry by 6/1
Slate Circle	4/11-7/12	1/13-7/16	N/A		dry by 6/1	recorder data corrupted; dry by 5/5
Stone Farm	4/11-7/12	5/7-7/16	3/6-7/23		recorder malfunction until 5/7	
Swain	4/11-7/12	1/13-7/16	3/5-7/23		wet all year	wet all year
Tiger Pond	6/7-6/12	1/13-7/16	3/6-7/23	dry until 6/7; logger burned on 6/12	dry by 6/1	dry 5/15

Figure 17. Photograph of froglogger positioned on a dead tree in an enhancement wetland (Block T).

Dipnet Sampling

Amphibian larval sampling was conducted using D-frame dipnets at each site during set intervals (Figure 18). Dipnetting was conducted across all sites multiple times during the breeding season. Fall surveys were not done, because it was determined that fall breeders didn't not occur in the ranges of the wetland sites. Sampling involved 30 dipnet sweeps at each site, making sure to sample various portions of each wetland. Larval amphibians were identified and the number of larvae of each species was recorded. Field guides used include Conant and Collins (1998) and Gregoire (2005). Crother (2008) was used for nomenclature.

Dipnet samples were used to determine amphibian abundance. Total abundance of amphibian found on a site in each year was calculated using dipnet data and egg mass survey data (see Opportunistic Surveys) (Amphibian Mean Abundance Dipnet + Egg Mass Survey). Since an equivalent survey effort was put into each site, multiple times per year, the average total number of individuals recorded was used as amphibian mean abundance. Abundance calculations used estimates of equivalent number of adults for various life stages (see Appendix E).

Figure 18. Example of a D-frame dipnet used for amphibian sampling (photo from rickly.com).

Opportunistic Surveys

Opportunistic surveys were conducted at each site when acoustic dataloggers were installed and during each site visit when dipnet surveys were conducted. These surveys involved visual surveys for amphibians and/or their eggs, as well as listening for calling amphibians. Logs and other debris were lifted when possible to detect species that could be missed using other methods.

Sites were sampled for amphibians twice in 2013, and 4 times each in 2014 and 2015 (Table 5). Sites were sampled with dipnet surveys when water was present on the sites. Not all sites had adequate water for sampling in all months; for instance, in July 2015, six of the 16 sites were dry. However, there were some sampling events where all sites had water at the same time. All re-establishment sites had adequate water for sampling in all years.

Table 5. Log of dates of dipnet/opportunistic amphibian surveys on all sites and months when various sites were dry.

Dipnet/Opportunistic Amphibian Surveys			
	2013	2014	2015
Months Sampled	June July	March May June July	April May June July
	Dry in June - Block O **Dry in July** - Brandon's Pond	**Dry in March** - Swain Pond **Dry in May** - none **Dry in June** - Block O, Slate Circle **Dry in July** – Block O, Little Little Dismal, Pulpwood Pond, Slate Circle, Tiger Pond	**Dry in April** – none **Dry in May** – Block O, Pulpwood Pond, Slate Circle **Dry in June** – Block O, Little Little Dismal, Pulpwood Pond, Slate Circle **Dry in July** - Block O, Block T, Little Little Dismal, Pulpwood Pond, Slate Circle, Tiger Pond

METRIC CALCULATIONS AND OTHER ANALYSES

Eighty-nine (89) metrics were calculated for analysis of the data, including landscape attribute metrics, rapid assessments, water quality and hydrology metrics, vegetation structure and species metrics, macroinvertebrate community metrics, and amphibian community metrics (Table 6). Appendix E contains detailed descriptions of each metric as well as how they were calculated.

As one of the amphibian community metrics, anuran species adult body size was calculated for each site. Adult body size information was obtained by taking the midpoint of the size range for each species given in Beane et al. (2010). This enabled assessment of species composition in terms of large, mid-sized, and small frog and toad species.

PAST statistical software (Hammer et al. 2001) SIMPER analysis was used to calculate the similarity percentage based on the Bray-Curtis similarity index (Bray and Curtis 1957) between different types of sites, in terms of macroinvertebrate taxa (family level) and abundance.

Canonical Correspondence Analysis (CCA) was used to reveal the influence of environmental variables on species assemblages for macroinvertebrates and amphibians. This analysis uses similarity indices to plot sites in a dimensional space according to how closely they resemble each other. Environmental variables are overlaid on the plot with lines representing the strength and direction of the influence. Nonparametric correlations were run to test which variables were related to any aspect of macroinvertebrate or amphibian communities (richness, abundance, biotic indices). Those variables that were significantly related to biotic communities were selected, and assessed for autocorrelation. Variables were dropped if they were highly correlated with each other (Spearman's $r>0.50$, $p<0.10$). The result was a selection of variables for input into the CCA which were related to biotic community metrics but not correlated with each other. Once the CCA was run with the selected variables, the model was examined to make sure the variance explained by the axes was better than random. If not, variables with less influence on assemblages were dropped until the overall model explained more than random variation.

Table 6. List of parameters calculated for analyses.

Landscape Attribute Metrics	Vegetation Metrics
Landscape Development Intensity Index (LDI) 1 mile	Vegetation Coverage by Vertical Stratum
Landscape Development Intensity Index (LDI) 500 meters	· 0-0.5m height
Distance to nearest natural wetland	· >0.5-1m height
Distance to nearest paved road	· >1-2m height
Distance to nearest dirt road	· >2-5m height
Distance to nearest road (paved or dirt)	· >5-15m height
Mean width of vegetated natural buffer	· >15-30m height
Assessment Area	· <30m height
Rapid Assessment Methods	Vegetation Coverage by Growth Form
NC Wetland Assessment Method (NCWAM)	· Filamentous or matt forming algae
Ohio Rapid Assessment Method (ORAM)	· Ground lichens
Water Quality and Hydrology Metrics	· Bryophytes (mosses and liverworts)
Mean pH	· Submerged aquatic vegetation (SAV)
Mean Specific Conductivity	· Floating aquatic vegetation
Mean Dissolved Oxygen	· Emergent vascular vegetation
Mean Water Temperature	· Shrubs and saplings (2 separate size classes)
Mean Water Depth	· Broadleaf small trees and large trees
Pool Size	· Lianas, vines, and epiphytes
Water Volume	· Coniferous trees
Percent Days with Water (Inundation Time)	Ground Surface Attributes and Woody Debris/Snags
Total Annual Precipitation	· Standing water with no vegetation (B)
Macroinvertebrate Metrics	· Standing water with SAVs, moss, floating aquatics or algae (C)
Percent of Total Taxa	· Standing water with herbaceous emergents (D)
· % taxa Coleoptera	· Standing water with woody emergents (E)
· % taxa Crustacea	· All standing water = B+C+D+E (from above)
· % taxa Diptera	· Exposed soil/sediment
· % taxa Ephemeroptera	· Broadleaf litter
· % taxa Ephemeroptera + Trichoptera	· Coniferous litter
· % taxa Hemiptera	· All litter (broadleaf + coniferous)
· % taxa Mollusca	· Dead woody debris <5 cm dbh
· % taxa Odonata	· Dead woody debris ≥5 cm dbh
· % taxa Trichoptera	· Standing dead shrubs and saplings <5 cm dbh
· % taxa Worms	· Standing dead snags ≥5 cm dbh
· % taxa Other Taxa (mainly ants, spiders, lepidopterans, nematodes, and springtails)	Dominant Plant Species Richness
Macroinvertebrate Richness	Dominant Plant Species Mean C
Macroinvertebrate Abundance	Dominant Plant Species Floristic Quality Assessment Index (FQAI)
Macroinvertebrate Density	Dominant Plant Species $FQAI_{cover}$
Macroinvertebrate Diversity (Shannon's H)	Dominant Plant Species Total Cover
Macroinvertebrate Species Evenness (Simpson's 1-D)	Percent Canopy Cover
Macroinvertebrate Biotic Index (MBI)	Upland Buffer - Cover by Vegetation Type
Percent Sensitive Taxa	· Herbaceous vascular vegetation and matt forming mosses
Percent Tolerant Taxa	· Small shrubs and saplings (<0.5m)
Amphibian Metrics	· Larger shrubs and saplings (≥0.5-5m)
Amphibian Richness (dipnet only and dipnet+froglogger)	· Broadleaf trees (≥5m)
Amphibian Mean Abundance	· Lianas, vines, and epiphytes
Amphibian Diversity (Shannon's H')	· Coniferous trees (all sizes)
Mean Amphibian Adult Body Size	Total Number of Strata in Wetland
Amphibian Quality Assessment Index (dipnet only and dipnet+froglogger)	Total Number of Height Classes Present
Other Metric	Total Number of Strata in Upland Buffer
Fish Presence	

Results

PROFILE OF CLOSED CANOPY REFERENCE WETLANDS

Landscape Attributes

The Landscape Development Intensity Index (LDI) (Brown and Vivas 2005) was calculated for a 500-meter buffer around each wetland and also for a 1-mile buffer around the wetlands. A one-mile distance was chosen based on an extensive literature search and consultation with experts for maximum dispersal distances of the amphibian species of the Coastal Plain (Table 2). Generally, development intensity within the 500-meter buffer of the reference wetlands was very low - nearly completely undisturbed by anthropogenic land use changes (Table 7). Development intensity within one mile of the wetlands varied from 1.3 (modified natural area) to 3.3 (levels similar to pastureland, pine plantations, and recently logged areas).

The 500-meter buffers of the closed canopy reference wetlands were also primarily natural land, with 5% more land associated with human uses than around the open canopy reference wetlands (Figure 19). The 1-mile area surrounding the closed canopy reference wetlands included more significant pine plantations, and included a small amount of severely impacted areas (high density development and industrial) (Figure 20). Within a mile of the closed canopy reference sites, the land was nearly 60% natural land, 86% vegetated (natural + planted), and approximately 15% impacted (clearcut, developed, or barren).

Table 7. Landscape Development Intensity Index (LDI) values for closed canopy reference wetland buffers.

Site Name	County	LDI - 500 m	LDI - 1 mile
Block O Pond	Richmond	1.2	3.3
Cypress Pond	Brunswick	2.7	2.9
Gum Pond	Carteret	1.2	1.4
Pulpwood Pond	Scotland	1.1	2.1
Mean LDI Value		1.5	2.4

Figure 19. Distribution of land cover types within the 500-meter buffer of closed canopy reference wetlands (all 4 sites combined).

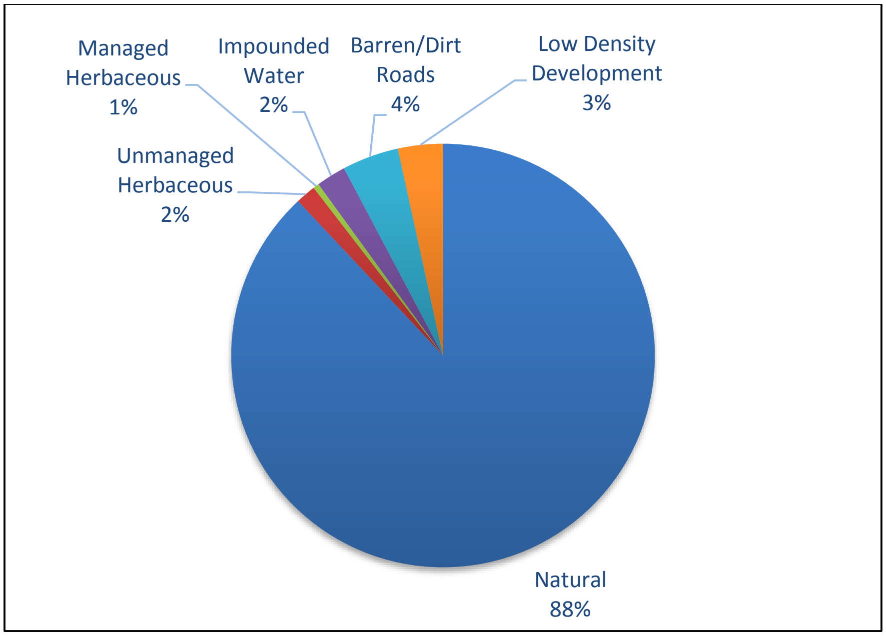
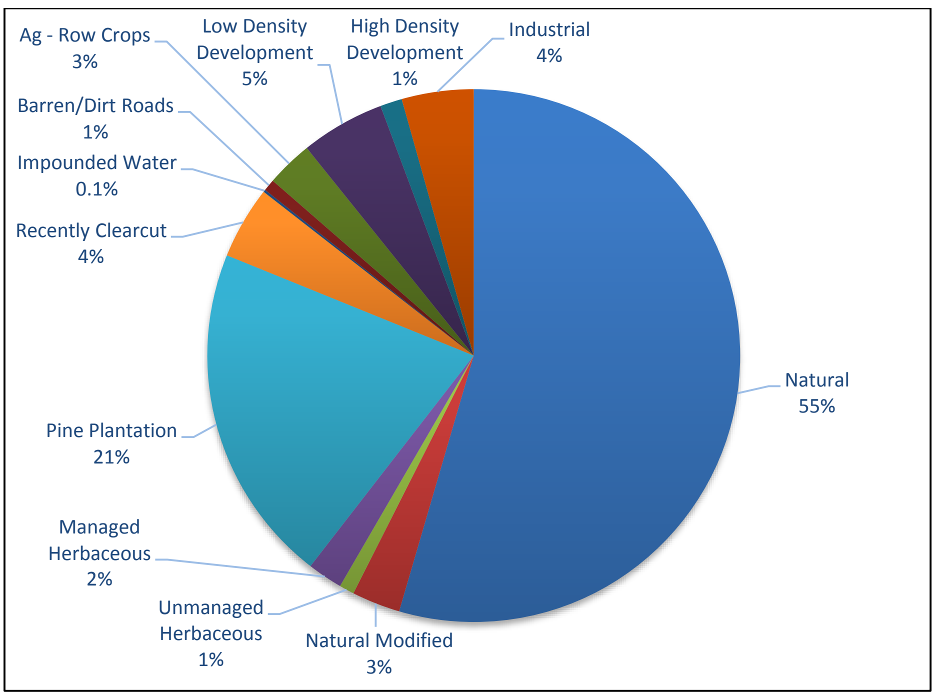


Figure 20. Distribution of land cover types within 1 mile of closed canopy reference wetland sites.



Rapid Assessments

All closed-canopy reference sites were rated high quality by the NCWAM, and superior by the ORAM (Table 8). They varied in total wetland size from 0.16 acre to nearly 2 acres.

Table 8. General information and rapid assessment results for closed-canopy reference sites.

USFWS = United States Fish and Wildlife Service; NC WRC = NC Wildlife Resources Commission; NHP = NC Natural Heritage Program; NCWAM = NC Wetland Assessment Method; ORAM = Ohio Rapid Assessment.

Site Name	County	Total Size (acres)	Ownership	NCWAM Type	NHP Type	NCWAM	ORAM Rating	ORAM Score (out of 90)
Block O Pond	Richmond	1.97	NC WRC	Basin	Upland Swamp Depression Forest	High	Superior	69
Cypress Pond	Brunswick	0.16	NC WRC	Basin	Small Depression Pond	High	Superior	81.5
Gum Pond	Carteret	1.18	USFWS	Non-Riverine Swamp Forest	Non-Riverine Swamp Forest	High	Superior	80
Pulpwood Pond	Scotland	0.62	NC WRC	Basin	Upland Swamp Depression Forest	High	Superior	69.5

Water Quality

Mean pH in the closed canopy reference wetlands was low, ranging from 3.5 to 4.4 (Table 9). Dissolved oxygen generally was low.

Table 9. Mean field water quality parameters by year on closed canopy reference sites.

(A meter malfunction prevented specific conductivity and dissolved oxygen data collection at Gum Pond in 2015).

	Mean pH			Mean Specific Conductivity (µS/cm)			WQ mean Dissolved Oxygen (mg/mL)		
Year	**2013**	**2014**	**2015**	**2013**	**2014**	**2015**	**2013**	**2014**	**2015**
Block O Pond	3.74	3.50	3.82	54.8	45.6	54.6	1.50	6.80	5.48
Cypress Pond	4.35	4.12	4.35	45.8	52.9	52.1	6.39	6.33	3.43
Gum Pond	4.19	3.86	4.15	57.9	75.9	-	2.88	2.63	-
Pulpwood Pond	3.82	3.96	3.92	19.5	24.4	20.4	2.90	6.70	5.71

Hydrology

Mean water depth at sampling points generally remained around one foot deep; sometimes closed canopy reference sites were dry (Figure 21). Volume showed large fluctuations at Block O site, in particular (Figure 22). All four sites showed a seasonal cycle of drying down (Figures 23-26). Cypress Pond was more responsive to precipitation events than the other sites. Fish were absent from all sites except Gum Pond, which had large amounts of topographical relief with pockets that probably stayed permanently wet.

Figure 21. Mean water depth at sampling points within closed canopy reference sites over time.

Two sites were not able to be measured for all dates.

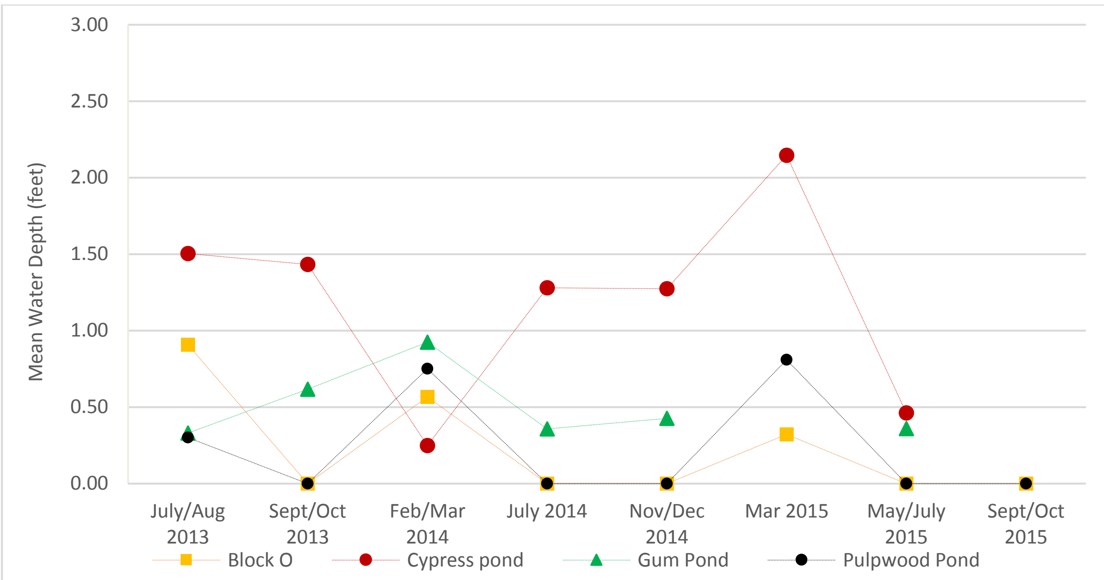


Figure 22. Pool volume for closed canopy reference sites over time.

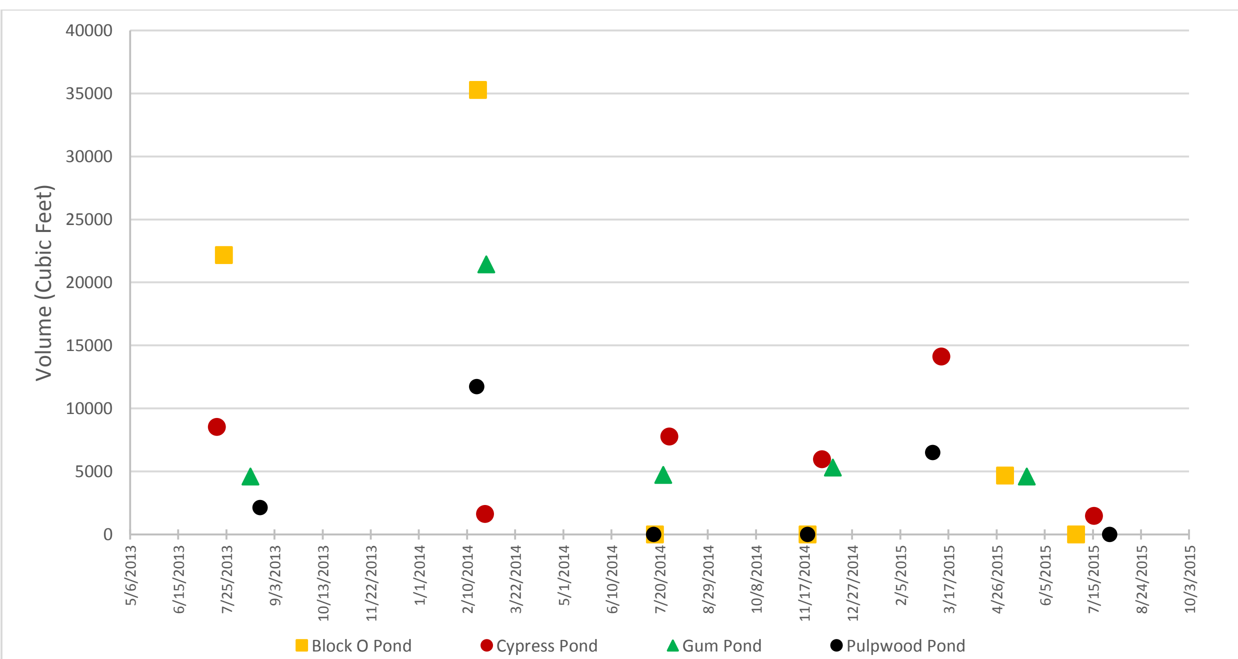


Figure 23. Hydrograph and precipitation data for Block O.

Water levels fell below level of detection below ground surface in the wetland between July 17 and November 16, 2014. Well was not deep enough to detect water levels below 2.2 feet underground. Dotted line represents ground surface level.

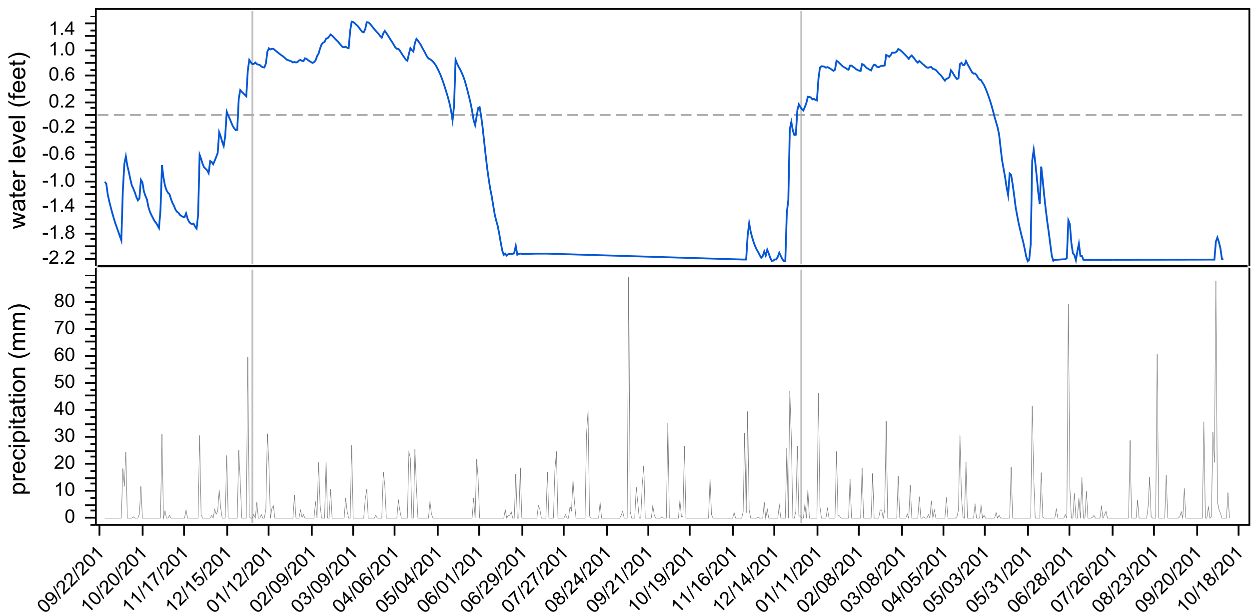


Figure 24. Hydrograph and precipitation data for Cypress Pond.

Water was always above ground level during study period. Well transducer was overtopped after July 2015.

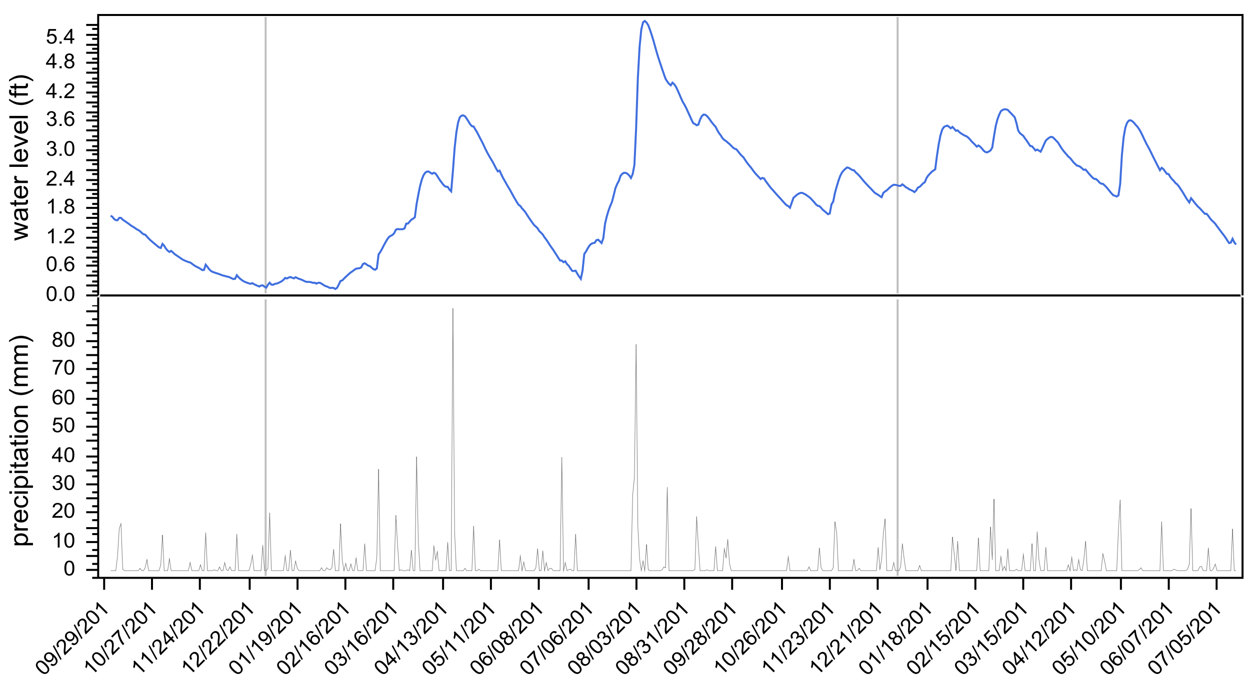


Figure 25. Hydrograph and precipitation data for Gum Pond.

Dotted line represents ground surface level.

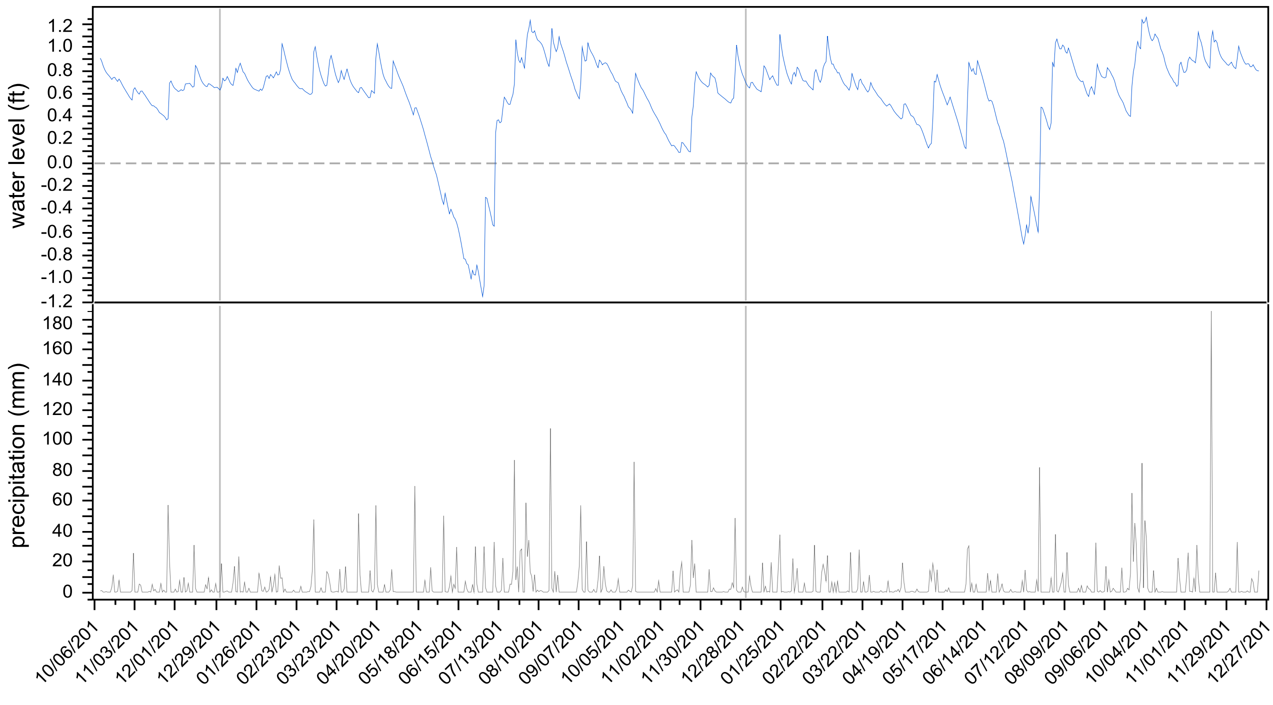
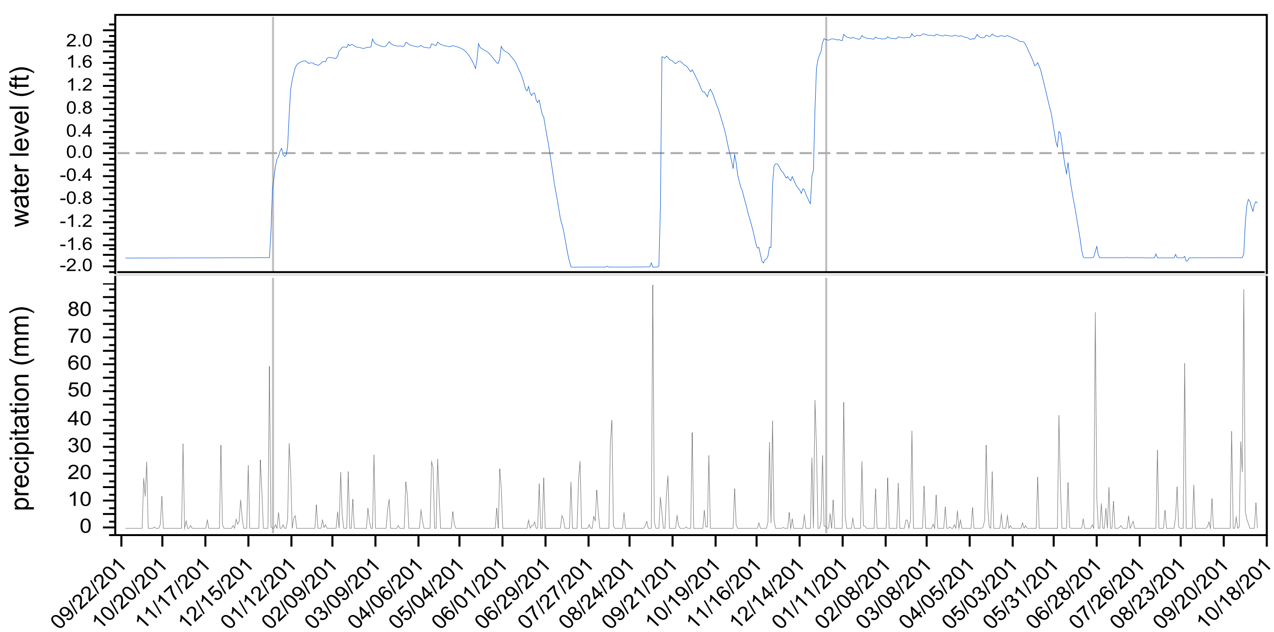


Figure 26. Hydrograph and precipitation data for Pulpwood Pond.

Well was not deep enough to detect water levels lower than 2.1 feet below surface. Dotted line represents ground surface level.



Vegetation

Three of the closed canopy reference sites were dominated by red maple (*Acer rubrum*) and swamp titi (*Cyrilla racemiflora*), along with a variety of shrub species. Cypress Pond was an exception, being dominated by pond cypress (*Taxodium ascendens*) trees and one herbaceous species (*Utricularia biflora*) (Table 10). The vegetation structure of the closed canopy reference sites was dominated by tall trees (>15m tall), followed by trees between 5 meters and 15 meters tall. Other size classes were represented in a minor way, if at all (Figure 27).

Upland buffer vegetation structure differed sometimes substantially between closed canopy reference sites (Figure 28). Block O's buffer was comprised mostly of coniferous trees, broadleaf trees, and vines. Cypress Pond buffer structure was more evenly distributed across structure types, without many vines. Pulpwood Pond was surrounded mainly by broadleaf and coniferous trees, with herbaceous species at ground level with few vines and shrubs. The buffer around Gum Pond was dominated by the shrubs of various sizes, coniferous trees, and herbaceous species. The upland areas surrounding Gum Pond were thinned and burned at the beginning of this study, which could explain the lack of broadleaf trees and vines.

Table 10. Dominant plant species in closed canopy reference wetlands.

Site	Scientific Name	Common Name	Growth Form
Block OPond	*Acer rubrum*	red maple	tree
	Cyrilla racemiflora	swamp titi	shrub
	Liquidambar styraciflua	sweetgum	tree
	Nyssa sylvatica	blackgum	tree
	Persea palustris	swamp bay	tree
	Pinus taeda	loblolly pine	tree
	Quercus nigra	water oak	tree
	Vaccinium corymbosum	highbush blueberry	shrub
	Woodwardia virginica	Virginia chainfern	herbaceous
Cypress Pond	*Taxodium ascendens*	pond cypress	tree
	Utricularia biflora	humped bladderwort	herbaceous
Gum Pond	*Acer rubrum*	red maple	tree
	Cyrilla racemiflora	swamp titi	shrub
	Ilex coriacea	large gallberry	shrub
	Lyonia ligustrina	maleberry	shrub
	Lyonia lucida	fetterbush lyonia	shrub
	Morella caroliniensis	southern bayberry	shrub
	Nyssa biflora	swamp tupelo	tree
Pulpwood Pond	*Acer rubrum*	red maple	tree
	Cyrilla racemiflora	swamp titi	shrub
	Erianthus sp.	plumegrass	tree
	Liquidambar styraciflua	sweetgum	tree
	Quercus nigra	water oak	tree
	Smilax rotundifolia	roundleaf greenbrier	vine

Figure 27. Wetland vegetation structure characterization in closed canopy reference wetlands averaged across years 1 and 3.

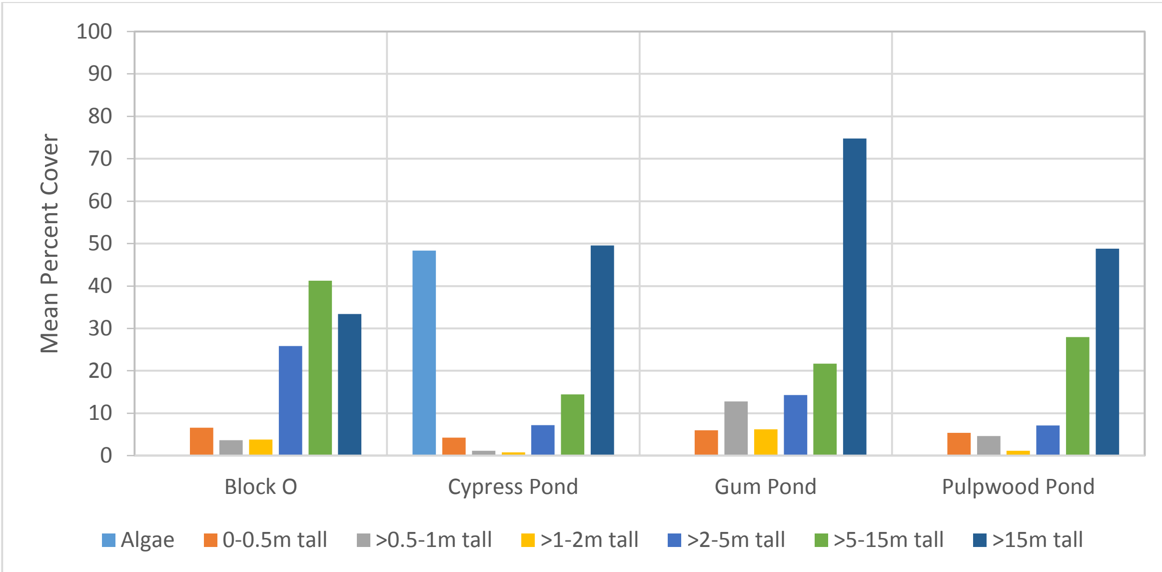
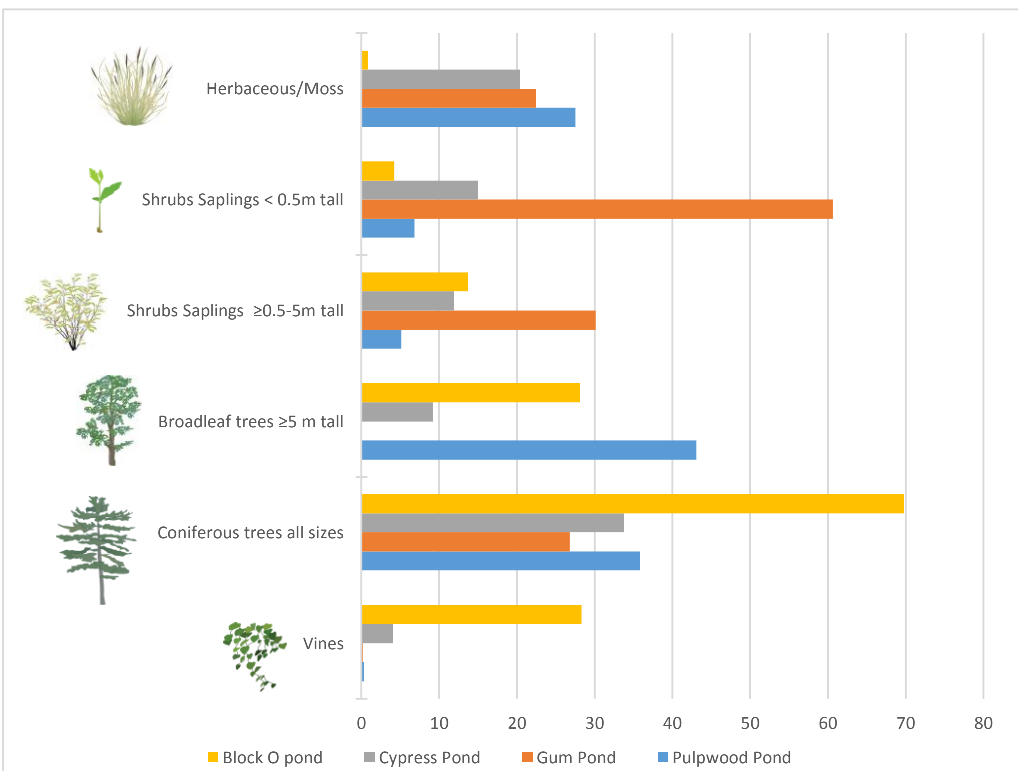


Figure 28. Upland buffer vegetation structure characterization in closed canopy reference wetlands averaged across years 1 and 3.

Bars represent mean percent cover in buffer plots.



Macroinvertebrates

Seventy-five (75) macroinvertebrate taxa were identified on closed canopy reference sites, among 4,028 individuals sampled (Table 11). Dipterans made up the majority (40-55% of total taxa per site) of the macroinvertebrate taxa on the closed canopy reference sites, followed by coleopterans, crustaceans, and odonates (Figure 29). "Other taxa" included mainly Hymenopterans, Arachnids, and Springtails (Class Collembola). Worms included aquatic and terrestrial types. Mayflies (Ephemeroptera), caddisflies (Trichoptera), and mollusks were absent from these sites. These closed canopy reference sites also showed greater fluctuations in the taxonomic representation of each group from the first year to the second than was seen on other sites types.

Figure 29. General taxonomic composition of macroinvertebrates on closed canopy reference sites.

Standard error bars are shown.

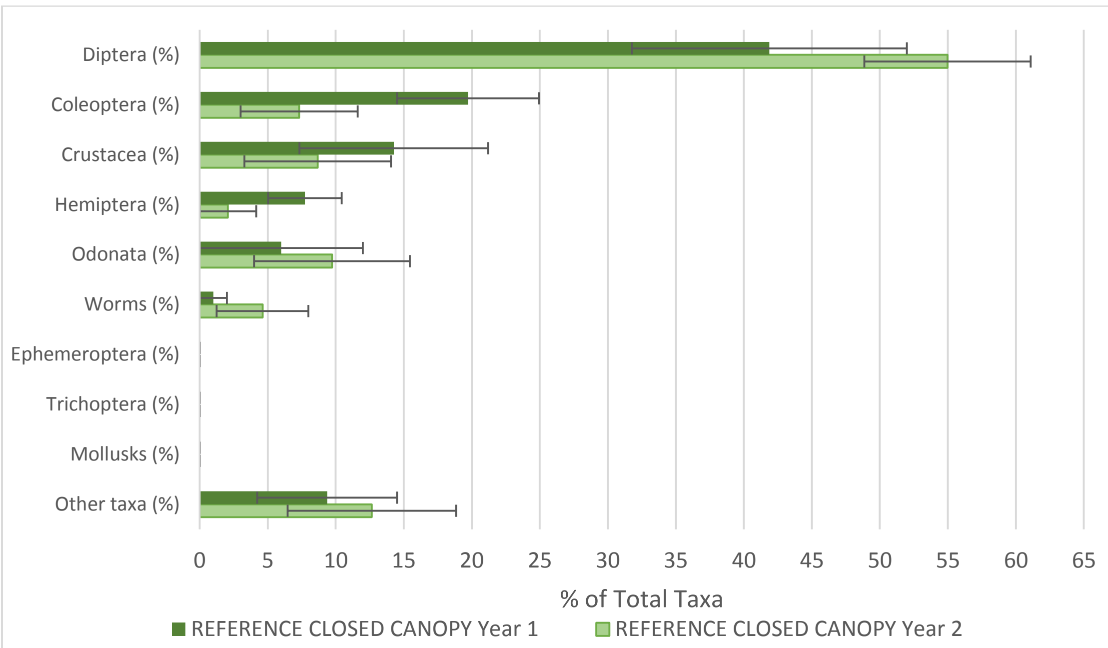


Table 11. Macroinvertebrate taxa found on closed canopy reference sites using sweep and bucket samples, years 1 and 2 combined.

Order - Family - Species	Frequency	Abundance	Taxon	Frequency	Abundance
Arachnida			Chironomidae (cont'd)		
Araneae	2	2	Limnophyes spp	2	2
Trombidiformes			Parachironomus chaetaolus	1	1
Hygrobatidae			Parachironomus tenuicaudatus	1	1
Hygrobates spp	1	4	Polypedilum illinoense gr	3	6
Class Collembola			Polypedilum tritum	5	290
Order Collembola	3	6	Psectrocladius flavus	1	5
Coleoptera			Smittia spp	4	21
Curculionidae	2	2	Culicidae		
Dytiscidae			Aedes spp	3	202
Acilius mediatus	1	1	Anopheles spp	1	1
Agabus stagninus	1	1	Culex spp	1	1
Bidessonotus spp	1	2	Orthopodomyia spp	1	1
Copelatus spp	1	2	Psorophora spp	1	12
Liodessus spp	1	1	Dolichopodidae	7	59
Neoporus spp	1	12	Empididae	2	3
Thermonectus basillaris	2	2	Muscidae		
Helophoridae			Limnophora spp	1	1
Helophorus spp	1	1	Terrestrial orange maggot	1	4
Hydrophilidae			Tiulidae		
Tropisternus collaris	1	1	Pseudolimnophila spp	1	2
Scirtidae			**Hemiptera**		
Scirtes spp	1	2	Corixidae	1	2
Terrestrial beetle	1	1	Sigara spp	2	10
Terrestrial elmid-like beetle	1	2	Gerridae	1	1
Crustacea			Notonectidae		
Amphipoda			Buenoa spp	1	1
Crangonyctidae			Notonecta spp	2	5
Crangonyx serratus	6	310	**Hymenoptera (ant)**	2	2
Crangonyx serricornis	1	12	**Insecta**		
Isopoda			**Archaeognatha**	1	1
Asellidae			**Megaloptera**		
Asellus spp	2	49	Corydalidae		
Caecidotea spp	1	15	Chauliodes rastricornis	1	1
Ostracoda			**Odonata**		
Ostracod	5	303	Aeshnidae		
Subclass Copepoda			Anax longipes	1	1
Copepod, unidentified	3	1847	Coenagrionidae		
Diptera			Enallagma spp	1	1
Ceratopogonidae			Ischnura spp	3	4
Ceratopogonidae (unid)	1	2	Libellulidae		
Dasyhelea spp	3	5	Erythemis simplicicollis	2	5
Palpomyia spp	1	2	Libellula incesta	1	3
Chaoboridae			Pachydiplax longipennis	1	1
Chaoborus spp	2	17	Tramea onusta	2	3
Mochlonyx cinctipes	1	2	Tramea spp	1	4
Mochlonyx spp	1	2	**Worms**		
Chironomidae			**Haplotaxida**		
Acamptocladius spp	1	9	Enchytraeidae	1	1
Camptocladius spp	1	556	Lumbriculidae	1	2

Order - Family - Species	Frequency	Abundance	Taxon	Frequency	Abundance
Chironomidae (unid)	1	3	Naididae		
Chironomus ochreatus	1	1	Aulodrilus pluriseta	1	1
Chironomus spp	9	95	**Phylum Nematoda**		
Dicrotendipes nervosus	1	1	Roundworm		
Gymnometriocnemus spp	1	2	Nematode	4	68
Kiefferulus dux	2	6	**Phylum Nemertea**	1	1
Kiefferulus spp	1	17			

Amphibians

On closed canopy reference sites, 17 different species of amphibians were detected (14 in 2013, 14 in 2014, and 15 in 2015) (Table 12). Most were anurans (14 species), and 3 species were salamanders or newts. Many species (12 of 17) were detected all three years of the study. The following species were detected only one of the three years: the green treefrog (*Hyla cinerea*), the barking treefrog (*Hyla gratiosa*), and the Atlantic Coast slimy salamander (*Plethodon chlorobryonis*). The latter species is not particularly a wetland breeder, so wider searches around the wetland may have resulted in detection.

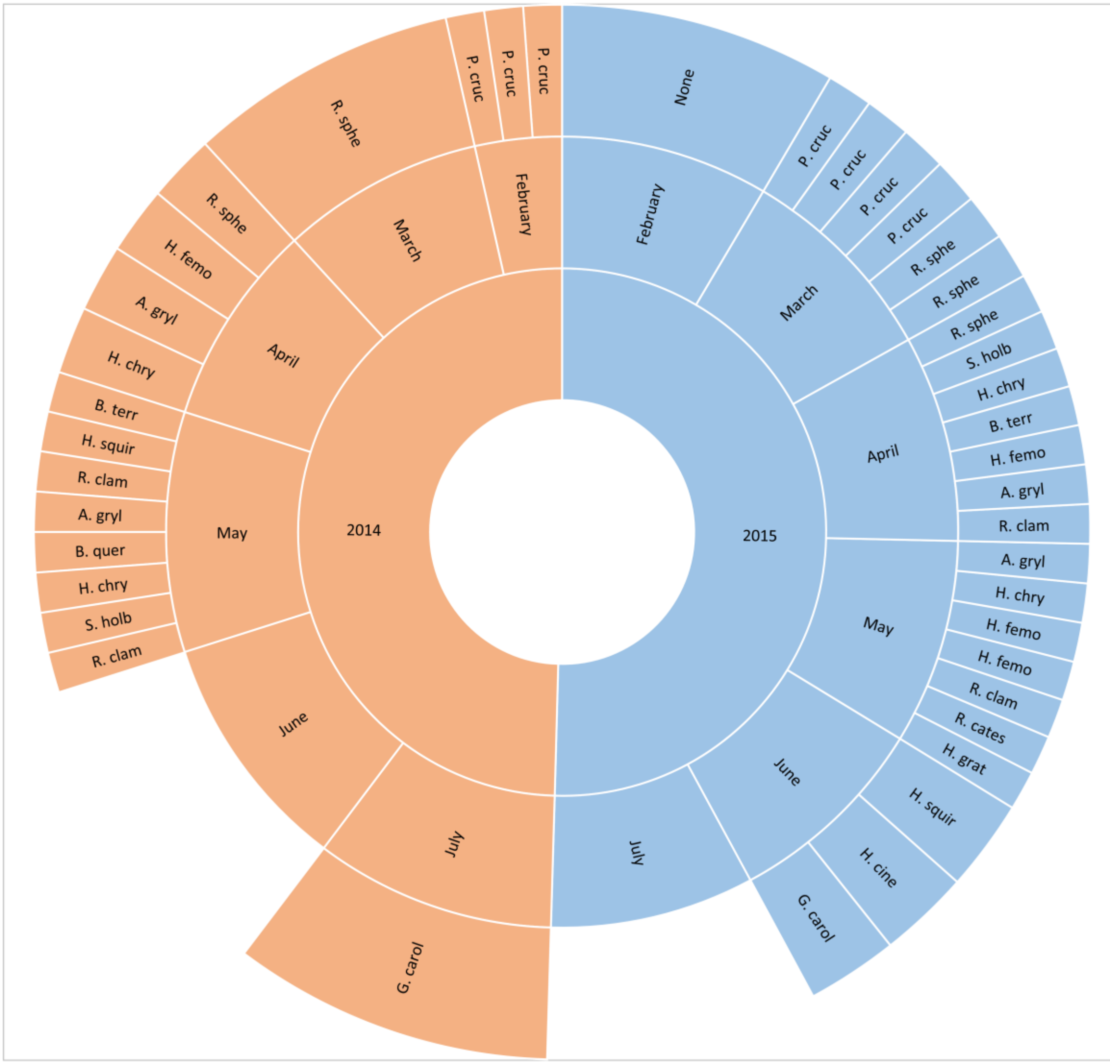
Anuran calling began earlier in 2014 than 2015 (frogloggers were installed too late in the season in 2013 to get first calling data) (Figure 30). Spring peepers (*Pseudacris crucifer*) were first to call at closed canopy reference sites in both years, as is typical for the species. They were followed by Southern leopard frogs (*Rana sphenocephala*). The Eastern Narrowmouth toad (*Gastrophryne carolinensis*) was the last species to initiate its calling.

Table 12. Amphibian species detected on closed canopy reference sites using observations with dipnets and egg mass surveys (O), and acoustic recordings with a froglogger (F).

Common Name	Species Name	2013	2014	2015	Total Years
Southern Cricket Frog	*Acris gryllus*	OF	OF	OF	3
Mabee's Salamander	*Ambystoma mabeei*	O	O	O	3
Oak Toad	*Bufo quercicus*	F	F		2
Southern Toad	*Bufo terrestris*	OF	F	F	3
Eastern Narrowmouth Toad	*Gastrophryne carolinensis*	OF	OF	OF	3
Cope's Gray Treefrog	*Hyla chrysoscelis*	OF	F	F	3
Green Treefrog	*Hyla cinerea*			F	1
Pinewoods Treefrog	*Hyla femoralis*	OF	OF	OF	3
Barking Treefrog	*Hyla gratiosa*			F	1
Squirrel Treefog	*Hyla squirella*		OF	OF	2
Eastern Newt	*Notophthalamus viridescens*	O	O	O	3
Atlantic Coast Slimy Salamander	*Plethodon chlorobryonis*	O			1
Spring Peeper	*Pseudacris crucifer*	F	OF	OF	3
American Bullfrog	*Rana catesbeiana*	F	O	F	3
Green Frog	*Rana clamitans*	OF	OF	OF	3
Southern Leopard Frog	*Rana sphenocephala*	OF	OF	OF	3
Eastern Spadefoot	*Scaphiopus holbrookii*	O	F	F	3

Figure 30. Months of first detection of calling species by frogloggers in the four closed canopy reference wetlands for 2014 and 2015.

Species are identified using the first letter of genus and first four letters of species name. Multiple mention of a species indicates separate sites within each year. Names appear in order of detection within a given year.



PROFILE OF RE-ESTABLISHMENT WETLANDS

Landscape Attributes

The Landscape Development Intensity Index (LDI) (Brown and Vivas 2005) was calculated for a 500-meter buffer around each study area, for re-establishment sites, and also for a 1-mile buffer around the study area. Generally, development intensity within the 500-meter buffer of the re-establishment wetlands was low but not unimpacted by human changes (Table 13). Development intensity within one mile of the wetlands varied from 1.9 (unmanaged herbaceous land) to 3.2 (levels similar to active pasture, pine plantations, and recently logged clearcuts).

The 500-meter buffers of the re-establishment wetlands were also primarily unmanaged herbaceous fields or natural land, with small amounts of pine plantation and roads (Figure 31). The 1-mile area surrounding the re-establishment wetlands was highly varied, but was dominated by unmanaged herbaceous land, natural land, pine plantations, and agricultural land (Figure 32).

Table 13. Landscape Development Intensity Index (LDI) values for open and closed canopy reference wetland buffers.

Site Name	County	LDI - 500 m	LDI - 1 mile
Dover Bay	Craven	1.7	3.1
Juniper Bay	Robeson	2.2	3.2
Parker Farms	Beaufort	2.1	1.9
Stone Farm	Brunswick	1.3	2.5
Mean LDI Value		1.8	2.7

Figure 31. Distribution of land cover types within 500-meter buffers of re-establishment wetlands.

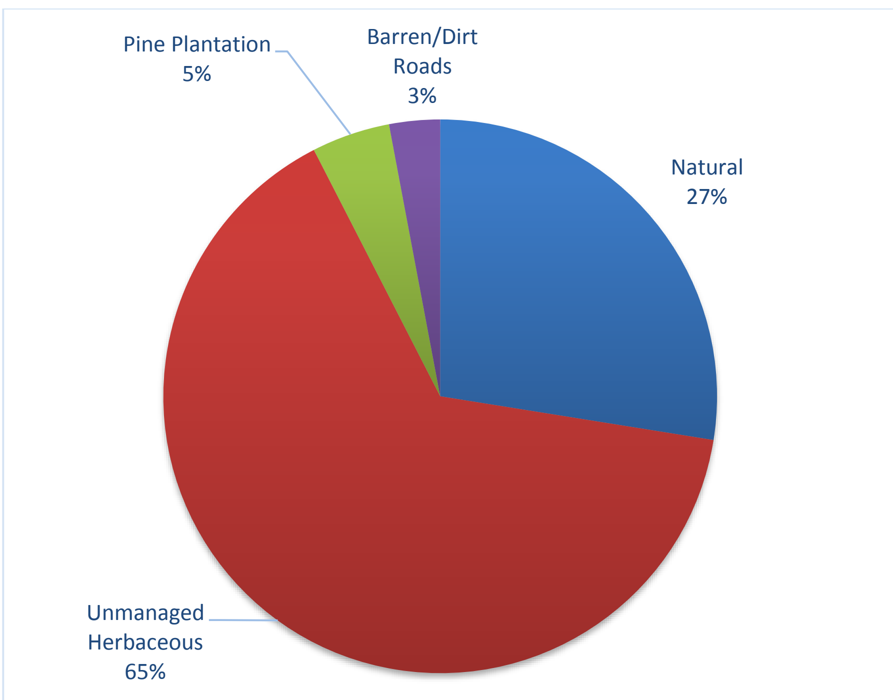
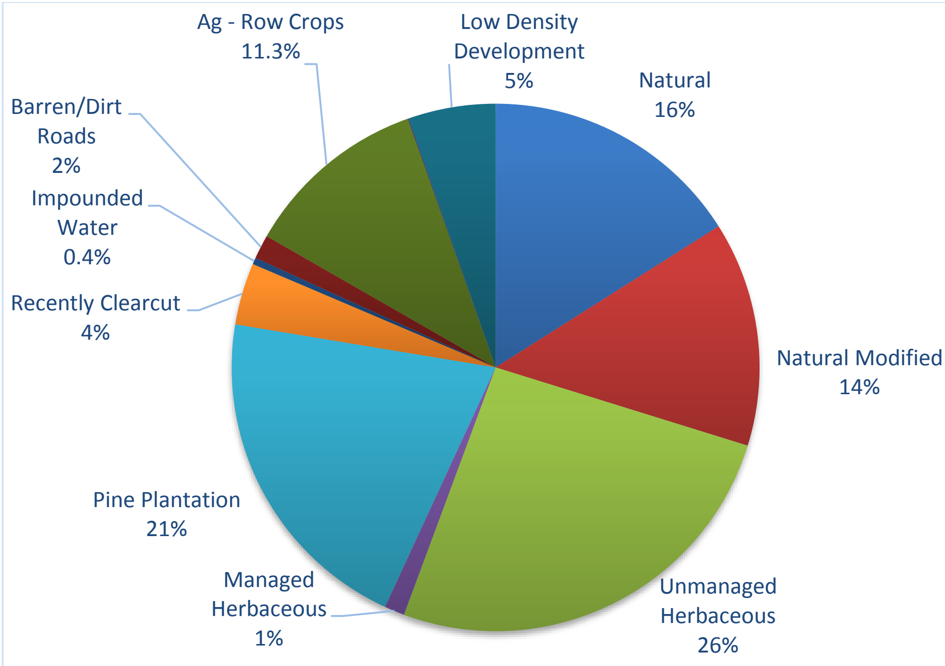


Figure 32. Distribution of land cover types within 1-mile buffers of re-establishment wetlands.



Rapid Assessments

All re-establishment sites were rated high quality by the NCWAM, and superior or moderate by the ORAM (Table 14). The assessment areas were portions of larger mitigation banks in each case, and varied in size from 0.69 acre to 3.5 acres (see maps in Appendix A). Only the assessment areas were rated using the rapid assessments.

Table 14. General information and rapid assessment results for re-establishment sites.

NCDOT = NC Dept. of Transportation; NC WRC = NC Wildlife Resources Commission; NHP = NC Natural Heritage Program; NCWAM = NC Wetland Assessment Method; ORAM = Ohio Rapid Assessment.

Site Name	County	Total Size (acres)	Ownership	NCWAM Type	NHP Type	NCWAM	ORAM Rating	ORAM Score (out of 90)
Dover Bay	Craven	2.63	NCDOT-WRC	Basin	Small Depression	High	Superior	64
Juniper Bay	Robeson	3.51	NCDOT	Basin	Vernal Pool	High	Moderate	48.5
Parker Farms	Beaufort	1.81	NCDOT	Hardwood Flat	Non-riverine Hardwood	High	Moderate	63.5
Stone Farm	Brunswick	0.69	NCDOT	Basin	Vernal Pool	Low	Moderate	54.5

Water Quality

Mean dissolved oxygen varied among sites and between years, but specific conductivity was less variable from year to year (Table 15). The fluctuating dissolved oxygen was potentially due to fluctuations in quantity of water on the sites, particularly at the water quality stations.

Table 15. Mean field water quality parameters by year on re-establishment sites.

	Mean pH			Mean Specific Conductivity (µS/cm)			WQ mean Dissolved Oxygen (mg/mL)		
Year	2013	2014	2015	2013	2014	2015	2013	2014	2015
Dover Bay	4.63	4.77	4.26	44.0	33.2	45.9	2.02	9.43	4.63
Juniper Bay	4.71	5.02	5.15	63.1	84.7	84.4	1.25	6.40	0.94
Parker Farms	4.09	4.60	4.67	60.5	47.3	43.5	3.30	3.07	4.73
Stone Farm	3.55	3.26	3.79	94.4	85	65.9	1.90	9.14	5.15

Hydrology

Water depths were shallow in the assessment areas of re-establishment sites (Figure 33). All sites showed a marked increase in volume in February of each year, except Stone Farm, where the assessment area of which was adjacent to a restored stream that drains the bay (Figure 34). This drainage moderated water levels within the assessment area of Stone Farm.

The assessment area of Dover Bay was permanently wet, but Juniper Bay, Parker Farms, and Stone Farm had areas (including where the wells were placed) which dried out each year (Figures 35 – 38). These conditions mimicked cycles in natural wetlands; however, connections to permanent water at all re-establishment sites meant fish were present at all sites.

Figure 33. Mean water depth at sampling points within re-establishment sites over time.

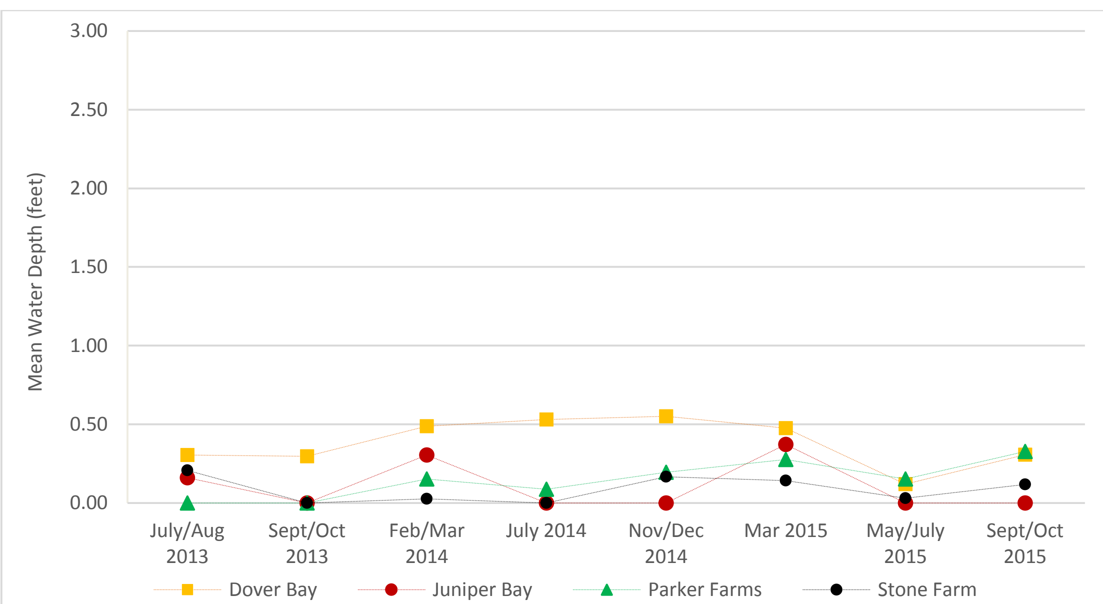


Figure 34. Pool volume for re-establishment sites over time.

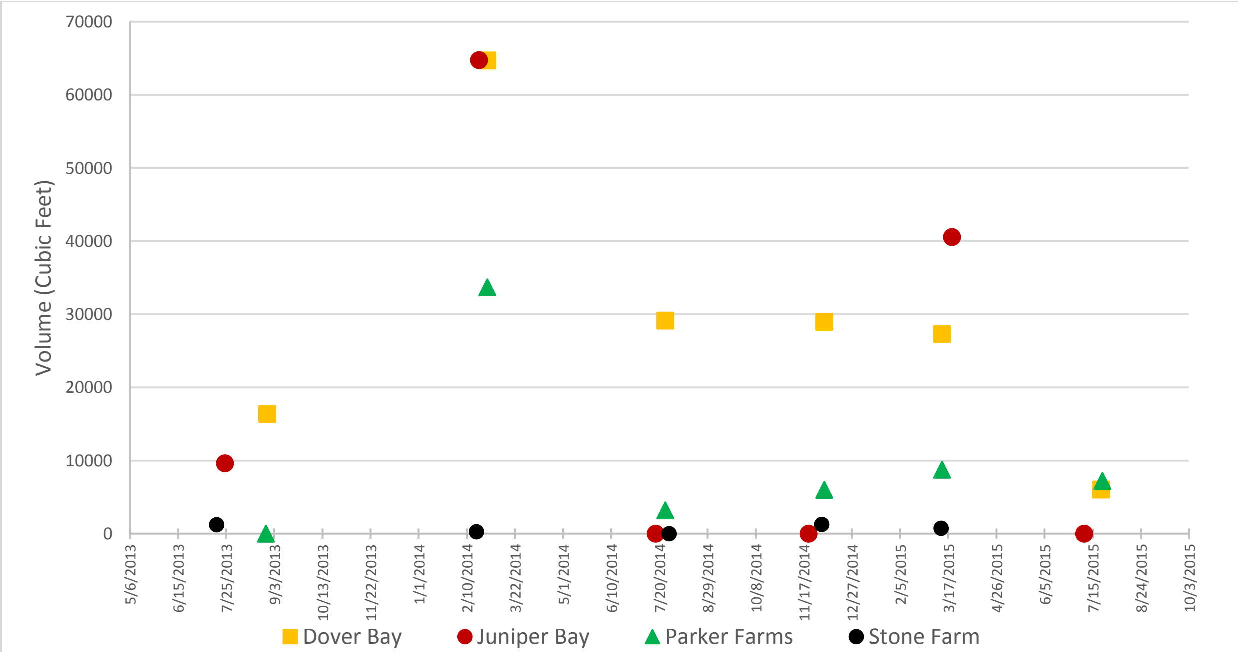


Figure 35. Hydrograph and precipitation data for Dover Bay.

Water was always above ground surface during study period.

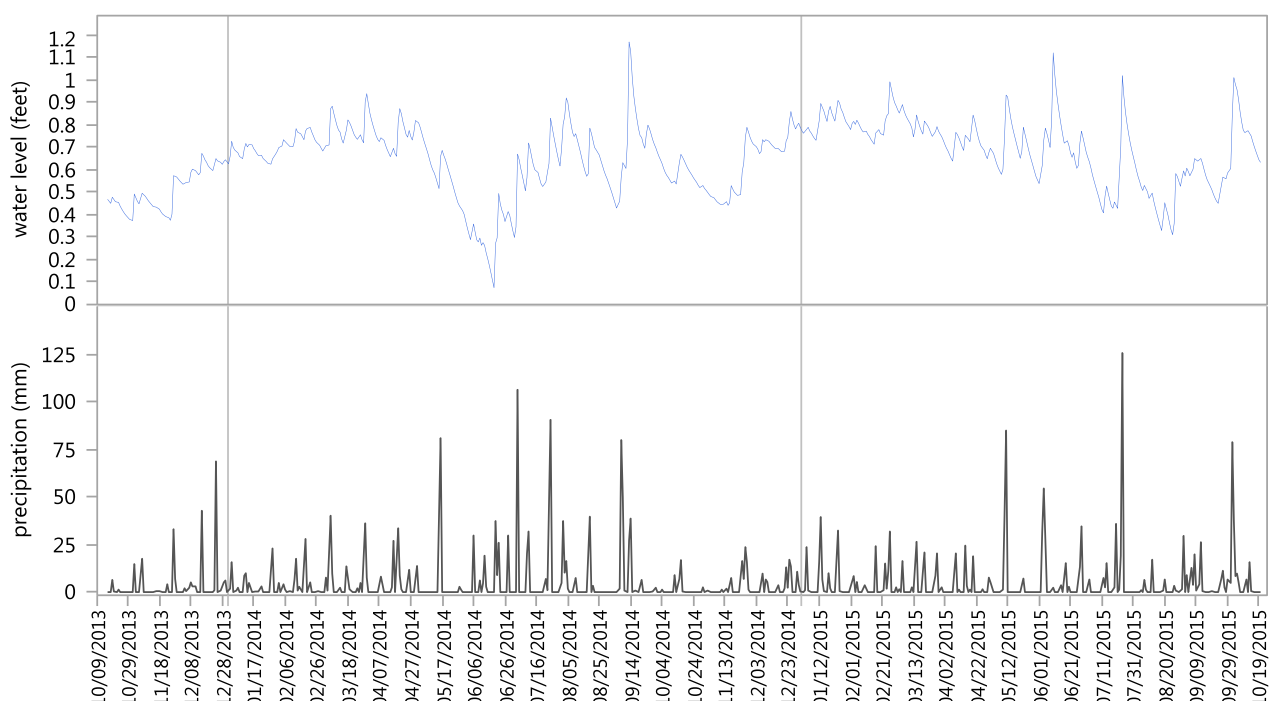


Figure 36. Hydrograph and precipitation data for Juniper Bay.

Because of recorder malfunction, no hydrology data were recovered between July 17 and Sept. 17, 2014 (double dash). Well was not deep enough to measure water levels lower than 2.1 feet below surface. Dotted line represents ground surface level.

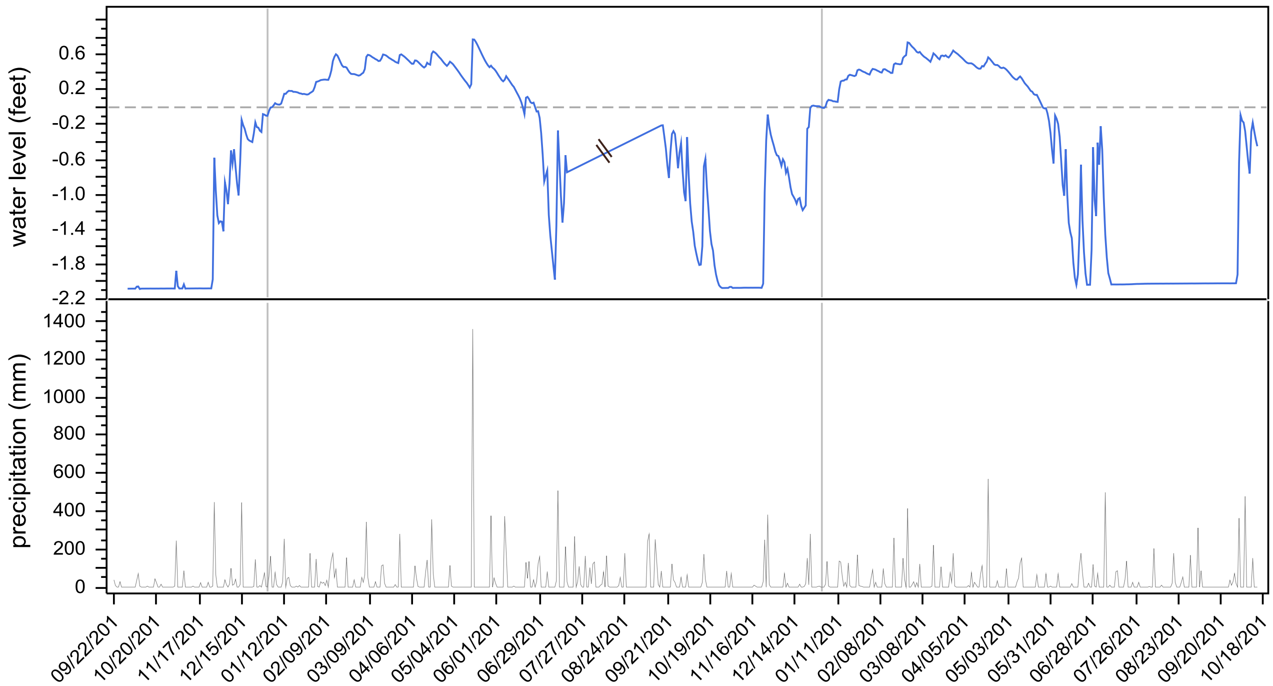


Figure 37. Hydrograph and precipitation data for Parker Farms.

Dotted line represents ground surface level.

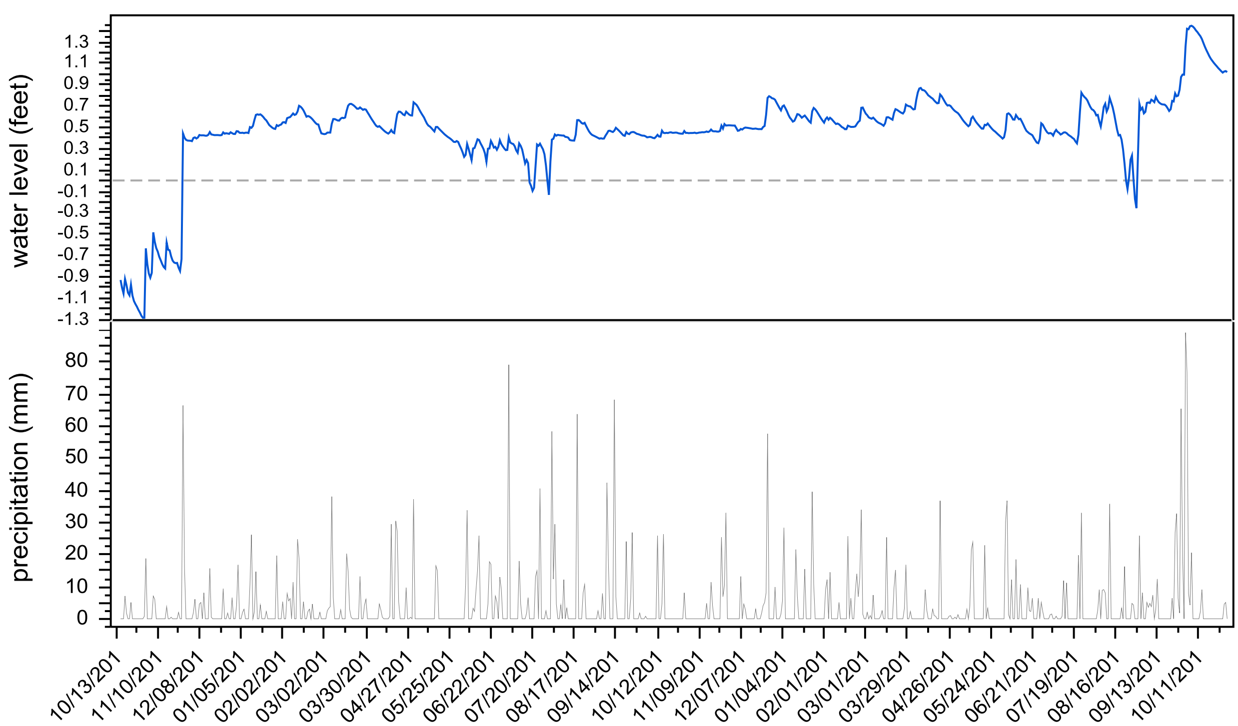
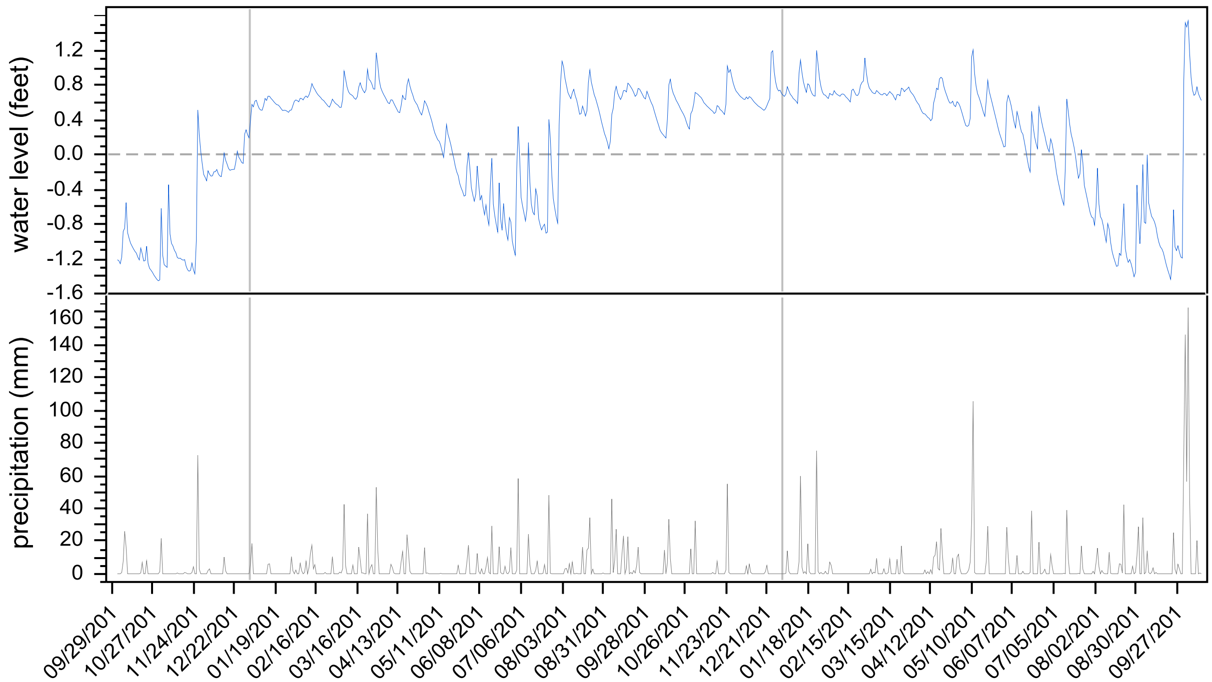


Figure 38. Hydrograph and precipitation data for Stone Farm.

Dotted line represents ground surface level.



Vegetation

In general, the assessment area section of the re-establishment sites were dominated (>10% species cover for woody species and >5% species cover for herbaceous species) by shrubby and herbaceous species, usually along with tree species (Table 16). Some areas outside the assessment areas appeared to have greater tree density than within the assessment areas. Parker Farms was the oldest re-establishment site, with planted trees >10 meters tall, and was dominated more by tree species than the other re-establishment sites, along with a few herbaceous species. The vegetation structure within the re-establishment wetlands was not consistent across all re-establishment sites (Figure 39). Tall trees (>15m tall) were absent from all sites except Parker Farms, the most mature re-establishment site. Low size classes comprised most of the vegetative cover on the other three re-establishment sites, but were minimal at the Parker Farms site.

Herbaceous cover was dominant in buffers around the re-establishment site sampling areas, but it declined somewhat at all sites between sampling years. Broadleaf shrubs comprised much of the woody vegetation in the buffers at two of the re-establishment sites, and conifers dominated the buffers at Juniper Bay (Figure 40). Upland buffer information at Parker Farms was not recorded, because wetland completely surrounded the sampling area.

Table 16. Dominant plant species in re-establishment reference wetlands.

Site	Scientific Name	Common Name	Growth Form
Dover Bay	*Acer rubrum*	red maple	tree
	Andropogon sp.	bluestem	herbaceous
	Carex sp.	sedge	herbaceous
	Juncus acuminatus	tapertip rush	herbaceous
	Sphagnum sp.	sphagnum	moss
	Woodwardia virginica	Virginia chainfern	herbaceous
Juniper Bay	*Andropogon sp.*	bluestem	herbaceous
	Baccharis halimifolia	eastern baccharis	shrub
	Juncus effusus	common rush	herbaceous
	Myrica cerifera	wax myrtle	shrub
	Pinus taeda	loblolly pine	tree
	Scirpus cyperinus	woolgrass	herbaceous
Parker Farms	*Alternanthera philoxeroides*	alligatorweed	herbaceous
	Boehmeria cylindrica	smallspike false nettle	herbaceous
	Nyssa aquatica	water tupelo	tree
	Quercus lyrata	overcup oak	tree
	Salix nigra	black willow	tree
	Scirpus cyperinus	woolgrass	herbaceous
	Taxodium distichum	bald cypress	tree
Stone Farm	*Andropogon glaucopsis*	purple bluestem	herbaceous
	Dichanthelium sp.	rosette grass	herbaceous
	Juncus effusus	common rush	herbaceous
	Liquidambar styraciflua	sweetgum	tree
	Rhynchospora harveyi	Harvey's beaksedge	herbaceous
	Rhynchospora inexpansa	nodding beaksedge	herbaceous
	Rubus argutus	sawtooth blackberry	shrub

Figure 39. Wetland vegetation structure characterization in re-establishment wetlands averaged across years 1 and 3.

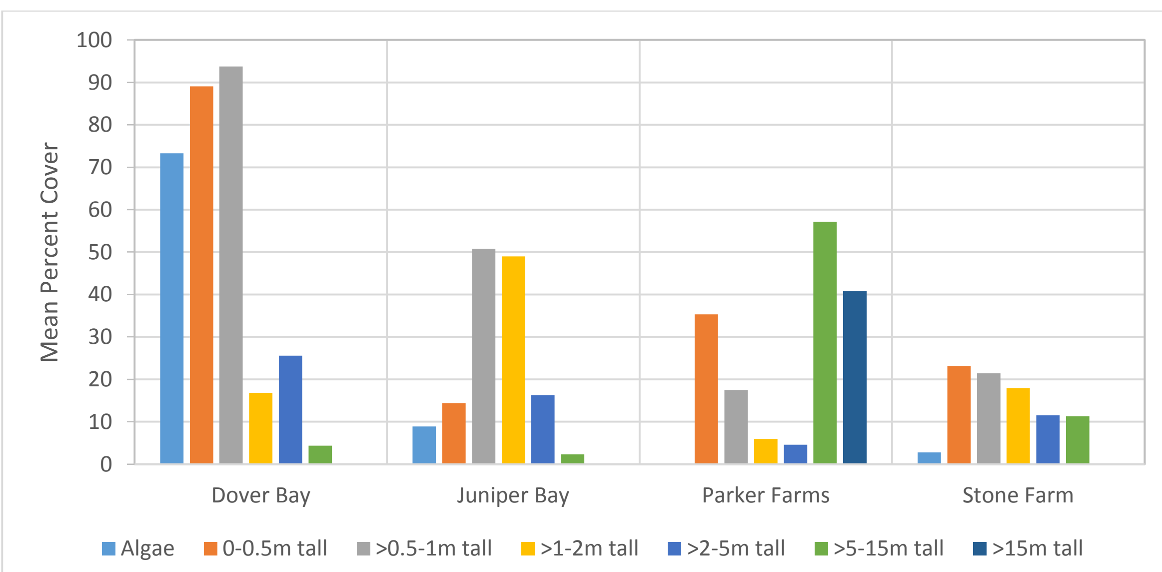
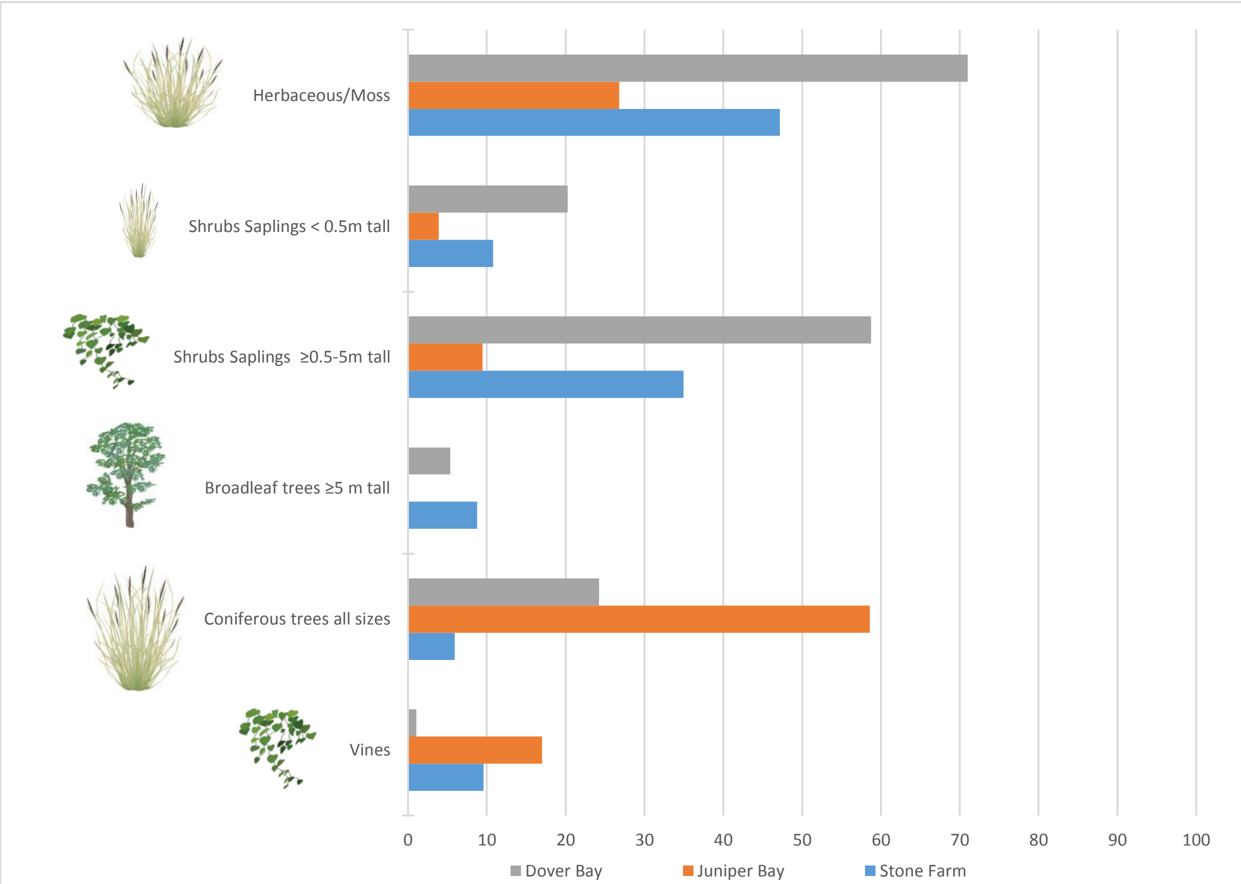


Figure 40. Upland buffer vegetation structure characterization in re-establishment wetlands averaged across years 1 and 3.

Bars represent mean percent cover in buffer plots. Upland buffer information at Parker Farms was not recorded, because wetland completely surrounded the sampling area.



Macroinvertebrates

One hundred thirty-three (133) macroinvertebrate taxa were collected on re-establishment sites, with a total of 3,308 individuals (Table 17). Dipterans made up the majority (nearly 45% of total taxa) of the macroinvertebrate taxa on the re-establishment sites, followed by coleopterans, and crustaceans (Figure 41). Re-establishment sites also notably contained four mollusk taxa, a group missing from nearly all other sites. "Other taxa" included mainly Hymenopterans, Arachnids, Lepidopterans, and Springtails (Class Collembola). Worms included only aquatic types. The mayflies (Ephemeroptera) and caddisflies (Trichoptera) were not absent, but together they represented less than 5% of the total taxa.

Figure 41. General taxonomic composition of macroinvertebrates on re-establishment sites in this study.

Standard error bars are shown.

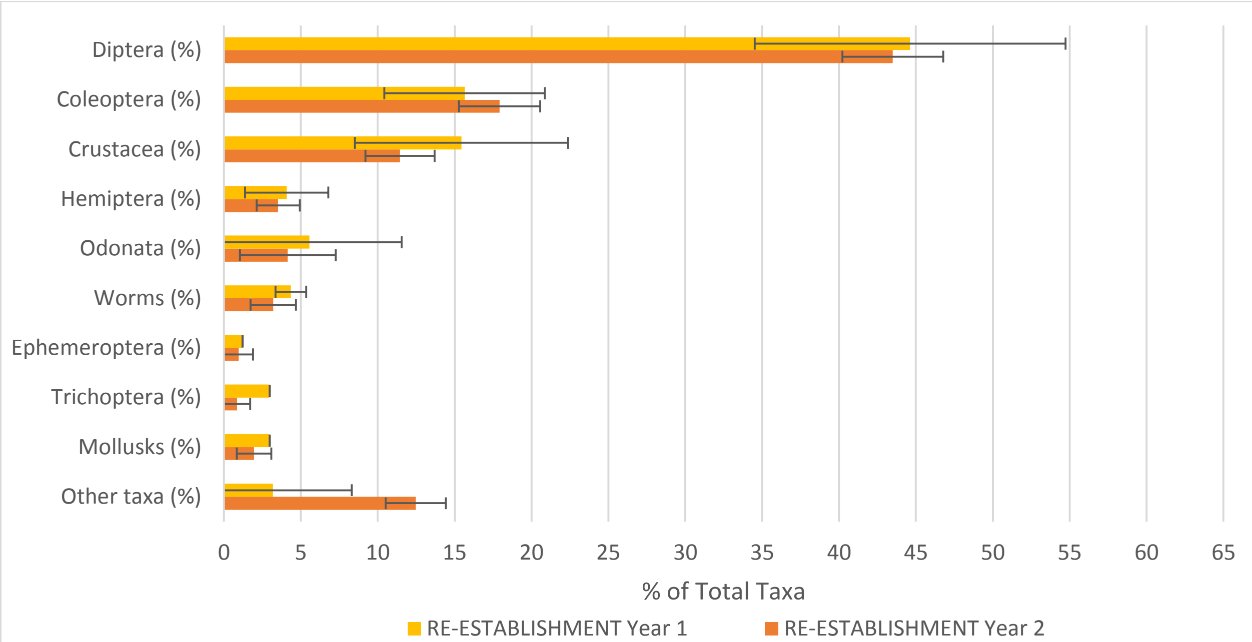


Table 17. Macroinvertebrate taxa found on re-establishment sites using sweep and bucket samples, years 1 and 2 combined.

Order - Family - Species	Freq.	Abund.	Taxon	Freq.	Abund.
Arachnida			Chironomidae (cont'd)		
Spider	5	14	Pseudosmittia spp	3	5
Pseudoscorpion	1	1	Sublettea coffmani	1	4
Trombidiformes			Tanypus carinatus	1	110
Hydrachnidiae			Tanypus spp	3	31
Hydracarina mite	1	1	Tanytarsus sp 1	2	7
Class Collembola (Springtails)			Tanytarsus sp 15	1	1
Order Collembola	4	10	Tanytarsus sp 2	1	1
Class Diplopoda (Millipede)			Tanytarsus sp 3	1	1
Millipede	1	4	Tanytarsus spp	1	1
Coleoptera			Tribelos spp	1	1
Dytiscidae			Zavreliella marmorata	1	1
Agabetes spp	1	2	Culicidae		
Agabus spp	6	95	Anopheles spp	3	4
Brachyvatus apicatus	1	1	Culex spp	1	3
Copelatus spp	1	3	Mosquitoe	1	7
Cybister spp	1	1	Psorophora spp	2	3
Hoperius planatus	2	2	Dolichopodidae	4	10
Hydaticus bimarginatus	1	1	Limoniidae		
Hydaticus spp	1	1	Limonia spp	1	2
Hydroporus spp	5	13	Ormosia spp	2	6
Laccophilus fasciatus	1	2	Midge		
Laccophilus spp	2	2	Midge pupae	1	1
Laccornis spp	2	4	Tabanidae		
Neoporus spp	2	7	Chrysops spp	5	6
Rhantus spp	3	9	Terrestrial white maggot	2	2
Thermonectus basillaris	2	6	Tipulidae		
Gyrinidae			Hexatoma spp	1	3
Dineutus spp	1	24	Unknown		

Order - Family - Species	Freq.	Abund.	Taxon	Freq.	Abund.
Gyrinus spp	1	1	Fly	1	1
Haliplidae			**Ephemeroptera**		
Haliplus spp	1	3	Baetidae		
Peltodytes spp	2	3	Callibaetis spp	1	11
Hydrochidae			Caenidae		
Hydrochus spp	1	1	Caenis spp	3	85
Hydrophilidae			**Hemiptera**		
Berosus spp	5	19	Belostomatidae		
Enochrus hamiltoni	1	7	Belostoma spp	2	2
Hydrochara spp	1	6	Corixidae		
Sperchopsis tessellatus	1	1	Sigara spp	5	7
Tropisternus spp	2	5	Trichocorixa spp	1	1
Noteridae			Nepidae		
Hydrocanthus oblongus	1	3	Ranatra spp	1	1
Sphaeriusidae			Notonectidae		
Sphaerius spp	1	1	Notonecta spp	2	5
Crustacea			**Hymenoptera (ant)**		
Amphipoda			Ant	2	7
Crangonyctidae			**Lepidoptera**		
Crangonyx serratus	1	4	Crambidae		
Crangonyx spp	11	495	Paraponyx spp	1	3
Decapoda			Moth/Butterfly, unidentified	2	2
Cambaridae			Pyralidae	2	17
Procambarus spp	3	14	**Mollusk**		
Unidentified Crayfish	1	1	**Class Gastropoda**		
Diplostraca			Slug	1	2
Daphniidae			**Basommatophora**		
Daphnia spp	2	18	Ancylidae		
Isopoda			Ferrissia spp	2	10
Asellidae			Lymnaeidae		
Asellus spp	8	628	Pseudosuccinea columella	2	19
Caecidotea spp	2	122	Planorbidae		
Ostracoda			Menetus dilatatus	2	7
Ostracod	11	528	**Odonata**		
Diptera			Coenagrionidae		
Ceratopogonidae			Enallagma spp	3	87
Bezzia/Palpomyia complex spp	5	10	Ischnura spp	2	22
Dasyhelea spp	2	2	Corduliidae		
Palpomyia spp	2	26	Epitheca costalis	2	4
Chironomidae			Lestidae		
Ablabesmyia janta	4	21	Lestes spp	2	2
Ablabesmyia parajanta group	1	1	Libellulidae		
Ablabesmyia peleensis	1	3	Celithemis spp	1	1
Chironomus spp	9	32	Ladona spp	2	2
Cladopelma spp	2	12	Libellula luciosa	1	3
Clinotanypus spp	3	10	Libellula spp	1	1
Constempellina brevicosta	1	1	Libellulidae unidentified	1	1
Corynoneura spp	2	28	Pachydiplax longipennis	4	64
Dicrotendipes leucoscelis	1	2	Sympetrum spp	2	2
Dicrotendipes modestus	4	5	**Trichoptera**		
Endochironomus nigricans	1	9	Hydropsychidae	1	1
Guttipelopia guttipennis	2	3	Hydroptilidae		
Gymnometriocnemus spp	4	4	Hydroptilid caddisfly	2	12
Kiefferulus dux	1	2	Orthotrichia spp	1	1
Kiefferulus spp	1	3	Oxyethira spp	1	1
Larsia spp	1	5	**Worms**		
Limnophyes spp	3	13	**Haplotaxida**		
Mesosmittia spp	2	4	Lumbriculidae	4	4
Parametriocnemus spp	1	1	**Phylum Nematoda**		
Paratanytarsus spp	2	8	Nematode	1	1
Paratendipes subaequalis	1	3	**Rhynchobdellida**		

Order - Family - Species	Freq.	Abund.	Taxon	Freq.	Abund.
Polypedilum halterale gr	3	49	Glossiphoniidae		
Polypedilum illinoense gr	7	23	Helobdella fusca	1	1
Polypedilum tritum	7	87	**Tubificida**		
Procladius spp	5	52	Naididae		
Psectrocladius (Monopsectrocladius) spp	1	1	Limnodrilus hoffmeisteri	1	53
Psectrocladius elatus	4	36	Nais spp	1	1
Psectrocladius flavus	1	4	Tubificidae no hair	3	103
Psectrocladius pilosus	1	26	Tubificidae with hair	1	1
Psectrocladius sordidellus	1	1			

Amphibians

On re-establishment sites, 13 different amphibian species were detected (11 in 2013, 11 in 2014, and 12 in 2015) (Table 18). Most were anurans (10 species), and one species was a newt. Many amphibian species (9 of 11) were detected all three years of the study on re-establishment sites. The Eastern newt (*Notophthalamus viridescens*) was the only species detected during only one year; all other species were detected at least two years of the study.

Anuran calling began earlier in 2014 than 2015 (frogloggers were installed to late in the season in 2013 to get first calling data) (Figure 42). Spring peepers (*Pseudacris crucifer*) and Southern leopard frogs (*Rana sphenocephala*) were the first species to call at re-establishment sites in both years, as is typical for these species. In 2014, the green frog (*Rana clamitans*) was the last species to initiate calling, while in 2015, the Eastern narrowmouth toad (*G. carolinensis*) was the last species to initiate calling (this species never called in 2014, but did call in 2013).

Table 18. Amphibian species detected on re-establishment sites using observations with dipnets and egg mass surveys (O), and acoustic recordings with a froglogger (F).

Common Name	Species Name	2013	2014	2015	Total Years
Southern Cricket Frog	*Acris gryllus*	OF	OF	OF	3
Southern Toad	*Bufo terrestris*	F	F	F	3
Eastern Narrowmouth Toad	*Gastrophryne carolinensis*	F		F	2
Green Treefrog	*Hyla cinerea*	F	F	F	3
Pinewoods Treefrog	*Hyla femoralis*	OF	O	OF	3
Squirrel Treefog	*Hyla squirella*	OF	F	OF	3
Eastern Newt	*Notophthalamus viridescens*		O		1
Spring Peeper	*Pseudacris crucifer*	F	F	F	3
Little Grass Frog	*Pseudacris ocularis*	F		F	2
American Bullfrog	*Rana catesbeiana*	F	OF	OF	3
Green Frog	*Rana clamitans*	OF	F	OF	3
Southern Leopard Frog	*Rana sphenocephala*	OF	OF	OF	3
Carpenter Frog	*Rana virgatipes*		F	OF	2

Figure 42. Months of first detection of calling species by frogloggers in the four re-establishment wetlands for 2014 and 2015.

Species are identified using the first letter of genus and first four letters of species name. Multiple mention of a species indicates separate sites within each year. Names appear in order of detection within a given year.

PROFILE OF OPEN CANOPY REFERENCE WETLANDS

Landscape Attributes

The Landscape Development Intensity Index (LDI) (Brown and Vivas 2005) was calculated for a 500-meter buffer around each wetland (or study area, for re-establishment sites) and also for a 1-mile buffer around the wetlands. Generally, development intensity within the 500-meter buffer of the reference wetlands was very low, nearly completely undisturbed by anthropogenic land use changes (Table 19). Development intensity within one mile of the wetlands varied from 1.3 (modified natural area) to 3.3 (levels similar to pastureland, pine plantations, and recently logged areas).

The 500-meter buffers of the open canopy reference wetland sites were primarily natural land with a few dirt roads and scattered homes (Figure 43). The 1-mile buffer surrounding open canopy reference wetlands was nearly 60% natural land, nearly 80% vegetated (natural + planted), and approximately 20% impacted (clearcut, developed, or barren) (Figure 44).

Table 19. Landscape Development Intensity Index (LDI) values for open canopy reference wetland buffers.

Site Name	County	LDI - 500 m	LDI - 1 mile
17 Frog Pond	Scotland	1.1	1.3
Brandon's Pond	Carteret	1.1	2.9
Swain Pond	Brunswick	2.5	3.0
Tiger Pond	Richmond	1.0	1.9
Mean LDI Value		1.4	2.3

Figure 43. Distribution of land cover types within the 500-meter buffer of open canopy reference wetlands (all 4 sites combined).

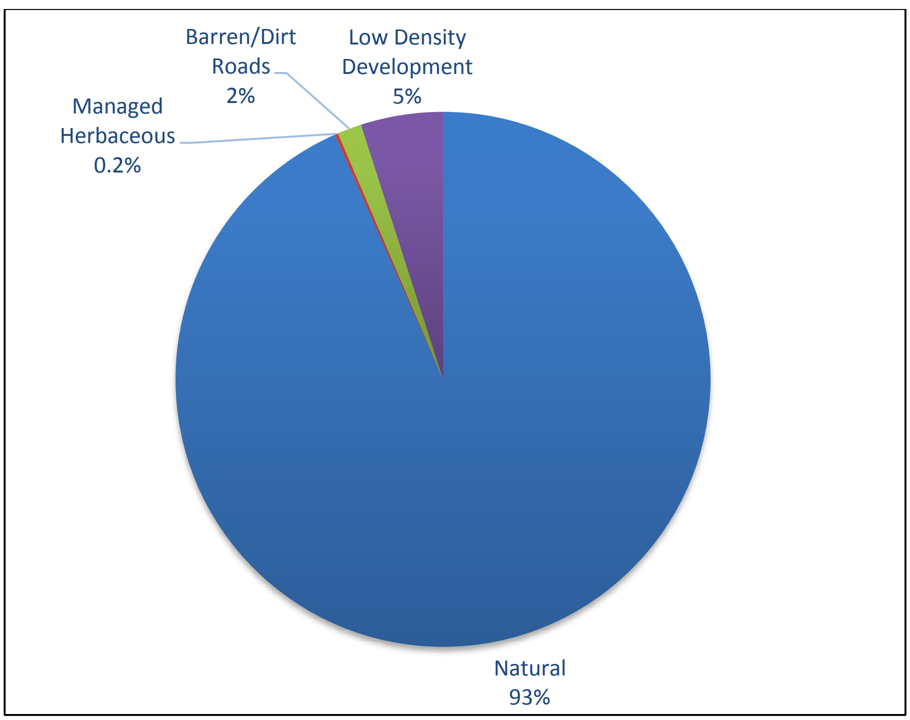
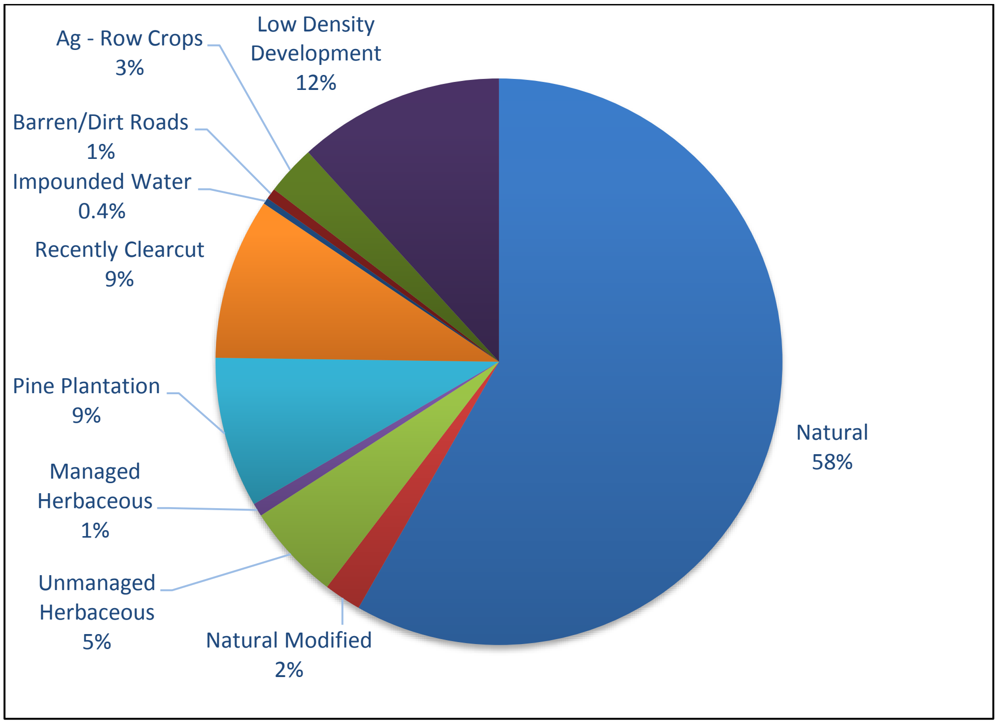


Figure 44. Distribution of land cover types within 1 mile of open canopy reference wetland sites.



Rapid Assessments

All open canopy reference sites were all rated high quality by the NCWAM, and superior by the ORAM (Table 20). They varied in total wetland size from 0.38 acre to 4.3 acres.

Table 20. General information and rapid assessment results for open-canopy reference sites.

NC WRC = NC Wildlife Resources Commission; USFWS = United States Fish and Wildlife Service; TNC = The Nature Conservancy; NHP = NC Natural Heritage Program; NCWAM = NC Wetland Assessment Method; ORAM = Ohio Rapid Assessment.

Site Name	County	Total Size (acres)	Ownership	NCWAM Type	NHP Type	NCWAM	ORAM Rating	ORAM Score (out of 90)
17 Frog Pond	Scotland	4.27	NC WRC	Basin	Vernal Pool	High	Superior	72.5
Brandon's Pond	Carteret	2.54	USFWS	Basin	Vernal Pool	High	Superior	69
Swain Pond	Brunswick	0.38	TNC	Basin	Small Depression	High	Superior	68.5
Tiger Pond	Richmond	0.39	NC WRC	Basin	Vernal Pool	High	Superior	67

Water Quality

The open canopy reference sites generally had low mean pH and moderately high dissolved oxygen (Table 21). Swain Pond had the highest specific conductivity of the open canopy sites, possibly because it was adjacent to a paved road and all other open canopy reference sites were surrounded by natural forested land.

Table 21. Mean field water quality parameters by year on open canopy reference sites.

(Meter malfunctioned at Brandon's Pond in 2015.)

Site	Mean pH			Mean Specific Conductivity (µS/cm)			WQ mean Dissolved Oxygen (mg/mL)		
	2013	2014	2015	2013	2014	2015	2013	2014	2015
17 Frog Pond	4.36	4.46	4.53	25.9	23.7	14.8	9.05	6.10	7.09
Brandon's Pond	3.69	3.38	3.21	39.9	38.7	-	8.90	9.56	-
Swain Pond	3.58	3.49	3.78	54.4	73.6	64.0	9.91	10.81	8.13
Tiger Pond	4.38	3.77	3.68	26.5	30.1	28.8	4.66	10.20	9.76

Hydrology

Total volume of open canopy reference wetlands was quite variable, in part due to the (large) size differences among the sites (Figure 45). Water depths also varied seasonally with Brandon's Pond reaching the greatest depth (fall 2014) (Figure 46). Tiger Pond held water for the shortest time of all open canopy reference ponds (Figures 47-50).

Figure 45. Mean water depth at sampling points within open canopy reference sites over time.

Two sites were not able to be measured for all dates.

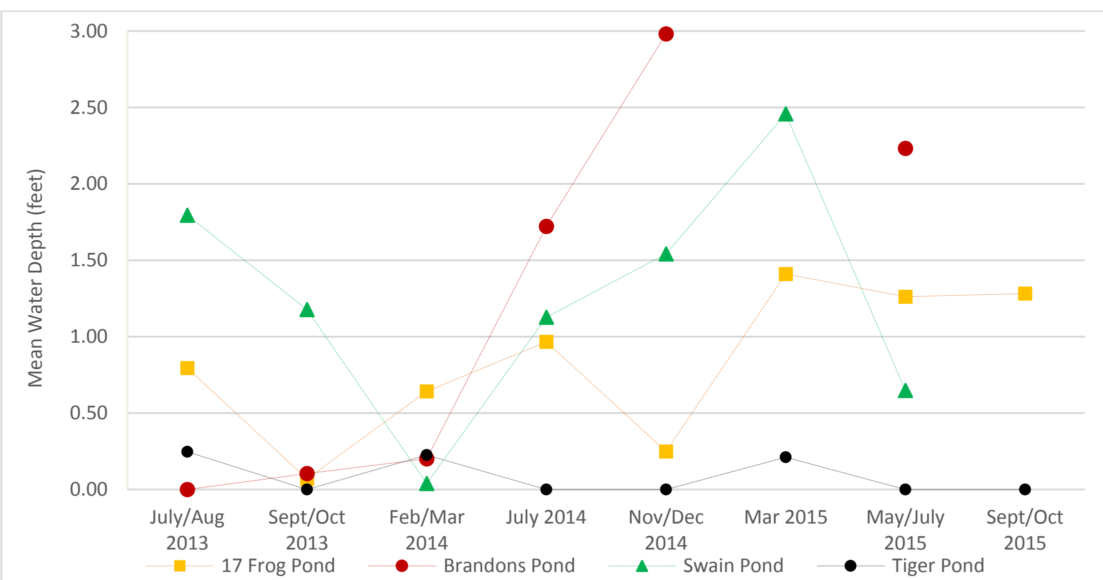


Figure 46. Pool volume in open canopy reference wetlands over time.

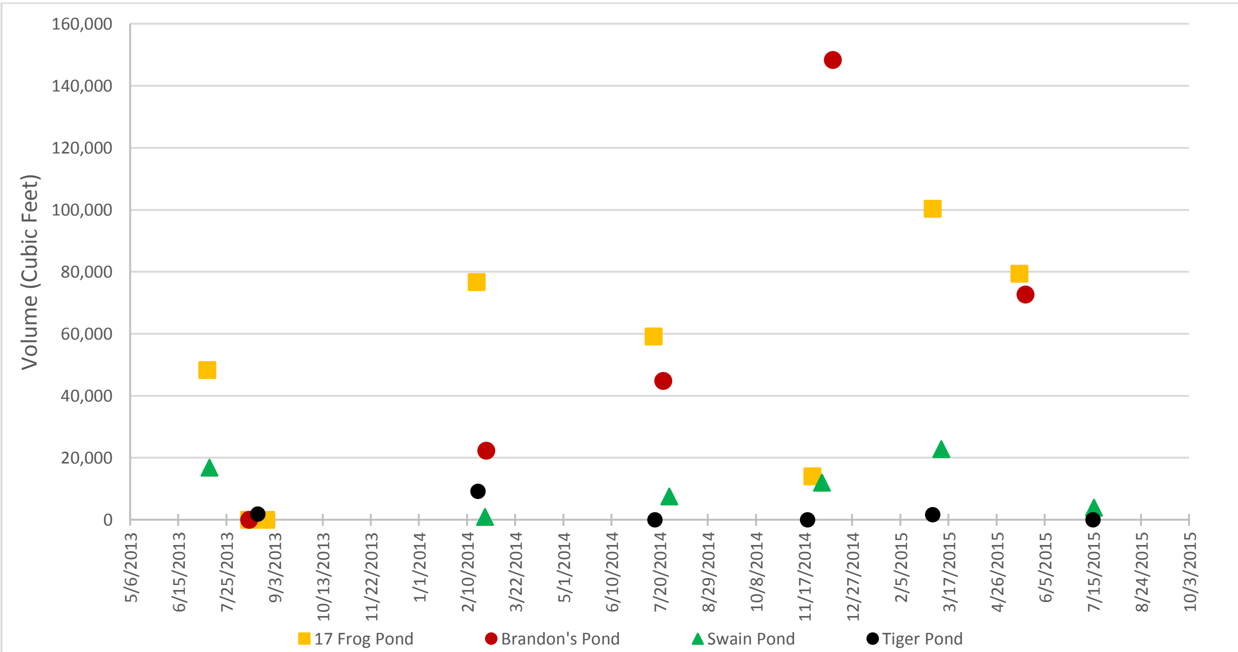


Figure 47. Hydrograph and precipitation data for 17 Frog Pond.

Dotted line represents ground surface level.

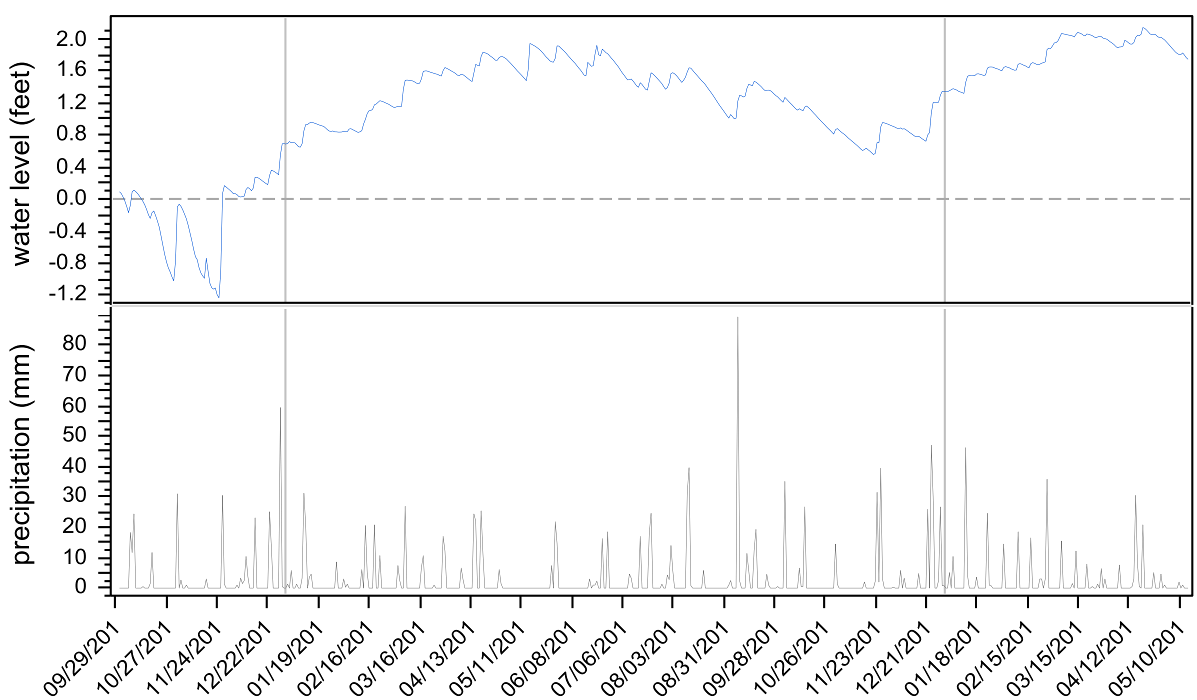


Figure 48. Hydrograph and precipitation data for Brandon’s Pond.

Well at Brandon’s Pond was overtopped by water level after February 26, 2014; transducer was replaced, but water levels rose above transducer again, so no successful data downloads have occurred since that date for Brandon’s Pond. Dotted line represents ground surface level.

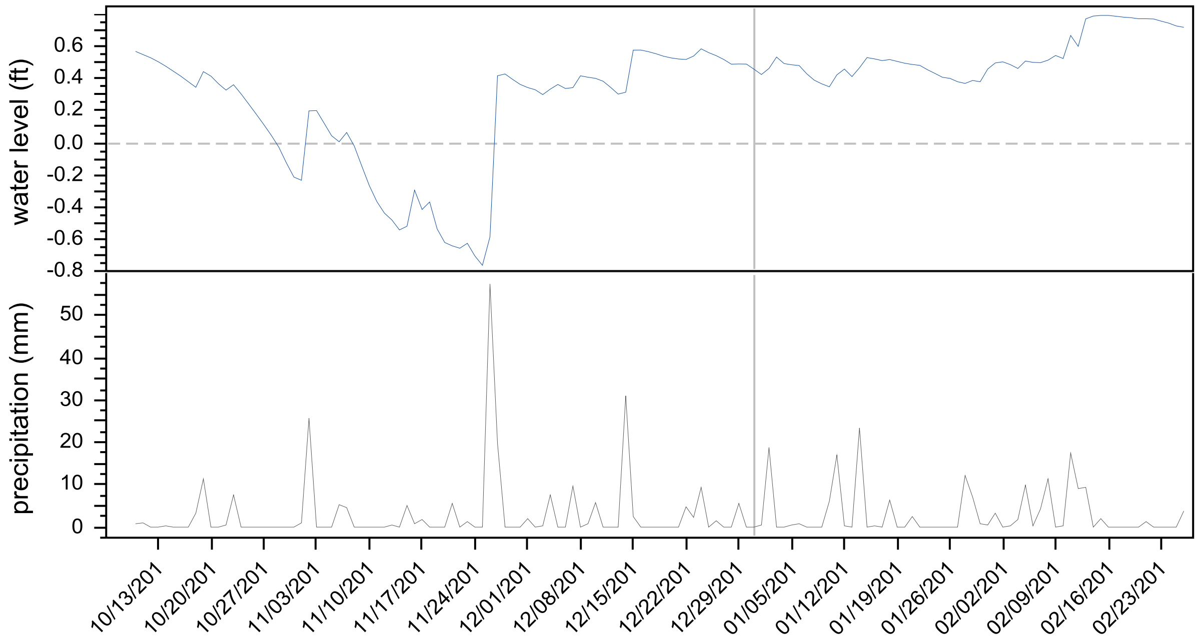


Figure 49. Hydrograph and precipitation data for Swain Pond.

Water was always above ground surface level during study period.

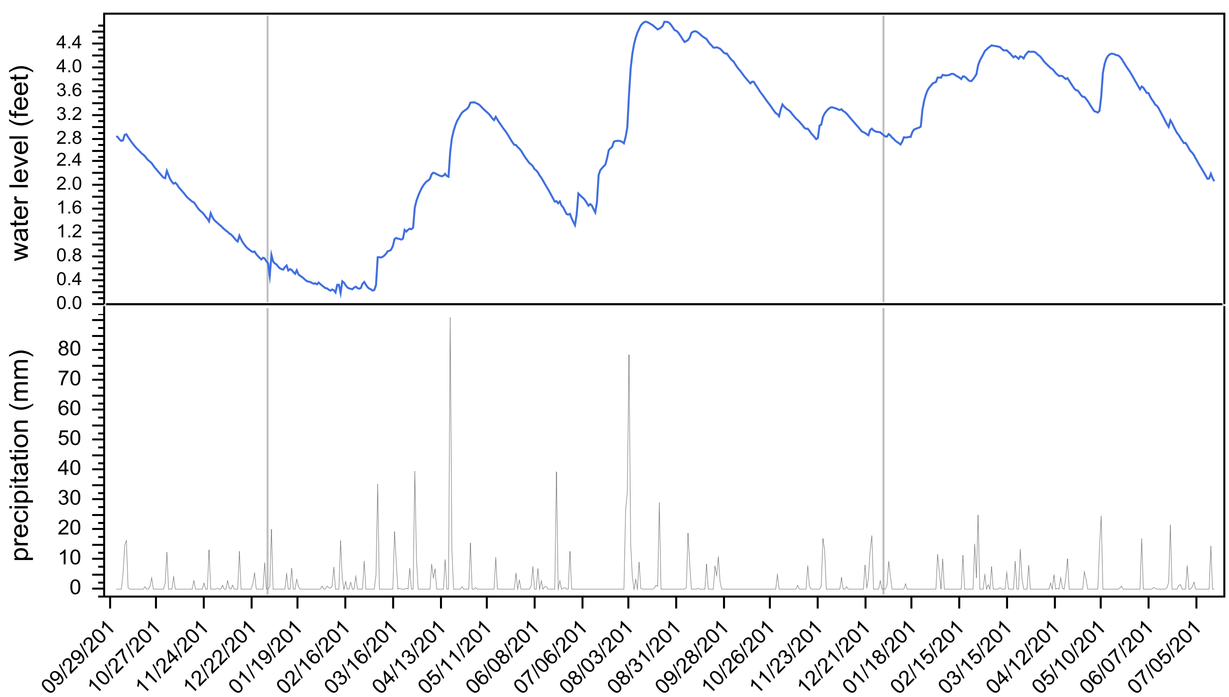
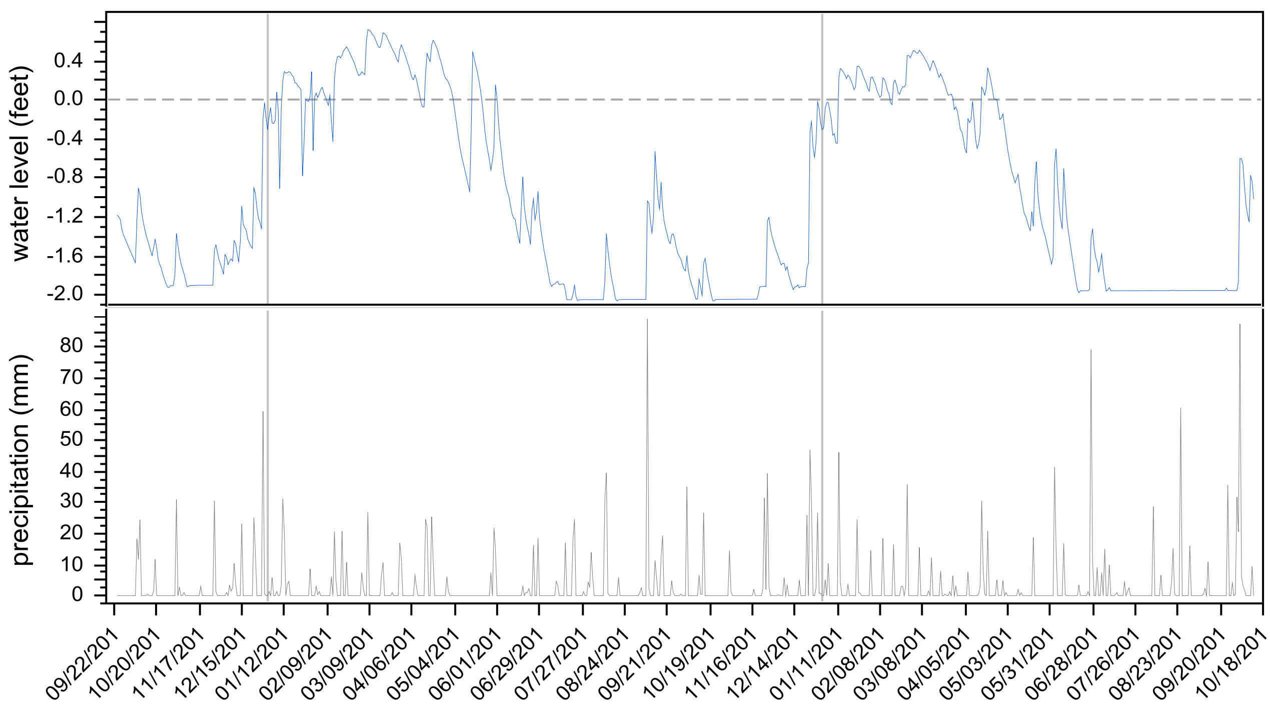


Figure 50. Hydrograph and precipitation data for Tiger Pond.

Dotted line represents ground surface level.



Vegetation

All open canopy reference sites were dominated by a herbaceous combination of forbs and grasses (Table 22). Swain Pond was the only site with a tree species (loblolly pine) which was invading the pond, but these mostly died out as water levels rose. Filamentous algae was a prominent part of the vegetative cover on three of four sites. Cover by tall trees was present in instances where large upland trees around the ponds were overhanging the wetlands, but usually not rooted in the wetland.

The vegetation structure on the open canopy reference sites was consistently dominated by low size classes (0 to 0.5m tall and 0.5m to 1.0m tall) (Figure 51). Trees were not present in the wetlands, except a small amount in Swain Pond.

Broadleaf trees and vines were largely absent from the upland buffers surrounding these open canopy sites (except at Swain Pond, which has not burned in recent years) (Figure 52). Coniferous trees and small shrubs were common in the upland buffers; buffers around Brandon's Pond had lower percent cover by pines than the other open canopy wetland sites.

The upland area surrounding 17 Frog Pond was burned in a controlled fire between year 1 and year 3; there was a large decrease in herbaceous cover in the buffer area between sampling years. This change may also have been due to seasonal shifts in cover by herbaceous plants as they seasonally cycle through growth and die-back (data were recorded later in the year 2013 [July] than 2015 [May]). Frequent controlled burning in this area around 17 Frog Pond has resulted in the lack of a shrub layer, no vines or broadleaf trees, and a dominance by coniferous pine trees.

The area surrounding Tiger Pond was also burned in a controlled burn between sampling years. Herbaceous species were probably being shaded or crowded out by small shrubs and saplings before the fire; after the fire, herbaceous cover was greater and small shrub cover was greatly reduced. Between sampling years, smaller shrubs/saplings grew up into larger shrubs/saplings and were not replaced by new small saplings (perhaps killed by fire). Vine cover in the buffer was also greatly reduced by the burning.

The upland area around Brandon's Pond was burned a year or two before the study began. The area around Swain Pond has not burned in recent years.

Table 22. Dominant plant species in open canopy reference wetlands.

Site	Scientific Name	Common Name	Growth Form
17 Frog Pond	*Andropogon sp.*	bluestem	herbaceous
	Centella asiatica	erect centella	herbaceous
	Eleocharis melanocarpa	blackfruit spikerush	herbaceous
	Eriocaulon compressum	flattened pipewort	herbaceous
	Leersia hexandra	southern cutgrass	herbaceous
	Rhexia mariana	Maryland meadowbeauty	herbaceous
	Sacciolepis striata	American cupscale	herbaceous
	Sagittaria isoetiformis	quillwort arrowhead	herbaceous
Brandon's Pond	*Andropogon capillipes*	chalky bluestem	herbaceous
	Lachnanthes caroliana	Carolina redroot	herbaceous
	Nymphaea odorata	American white waterlily	herbaceous
	Rhynchospora macrostachya	tall horned beaksedge	herbaceous
	Xyris sp.	yelloweyed grass	herbaceous
Swain Pond	*Juncus repens*	lesser creeping rush	herbaceous
	Pinus taeda	loblolly pine	tree
	Poaceae, unidentified	grass	herbaceous
	Sphagnum sp.	sphagnum	moss
	Zannichellia palustris	horned pondweed	herbaceous
Tiger Pond	*Andropogon glaucopsis*	purple bluestem	herbaceous
	Eleocharis sp.	spikerush	herbaceous
	Forb, unidentified	unidentified forb	herbaceous
	Ilex glabra	inkberry	shrub
	low grass, unidentified	unidentified grass	herbaceous
	moss	moss	moss
	Steinchisma hians	gaping grass	herbaceous

Figure 51. Wetland vegetation structure characterization in open canopy reference wetlands averaged across years 1 and 3.

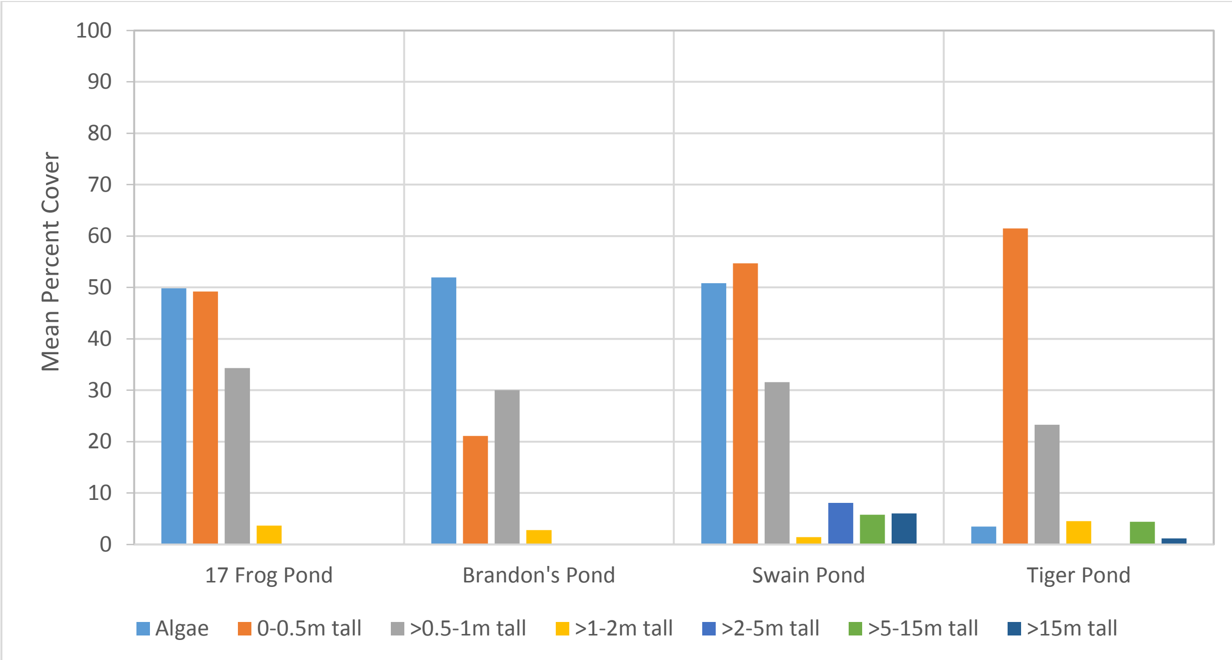
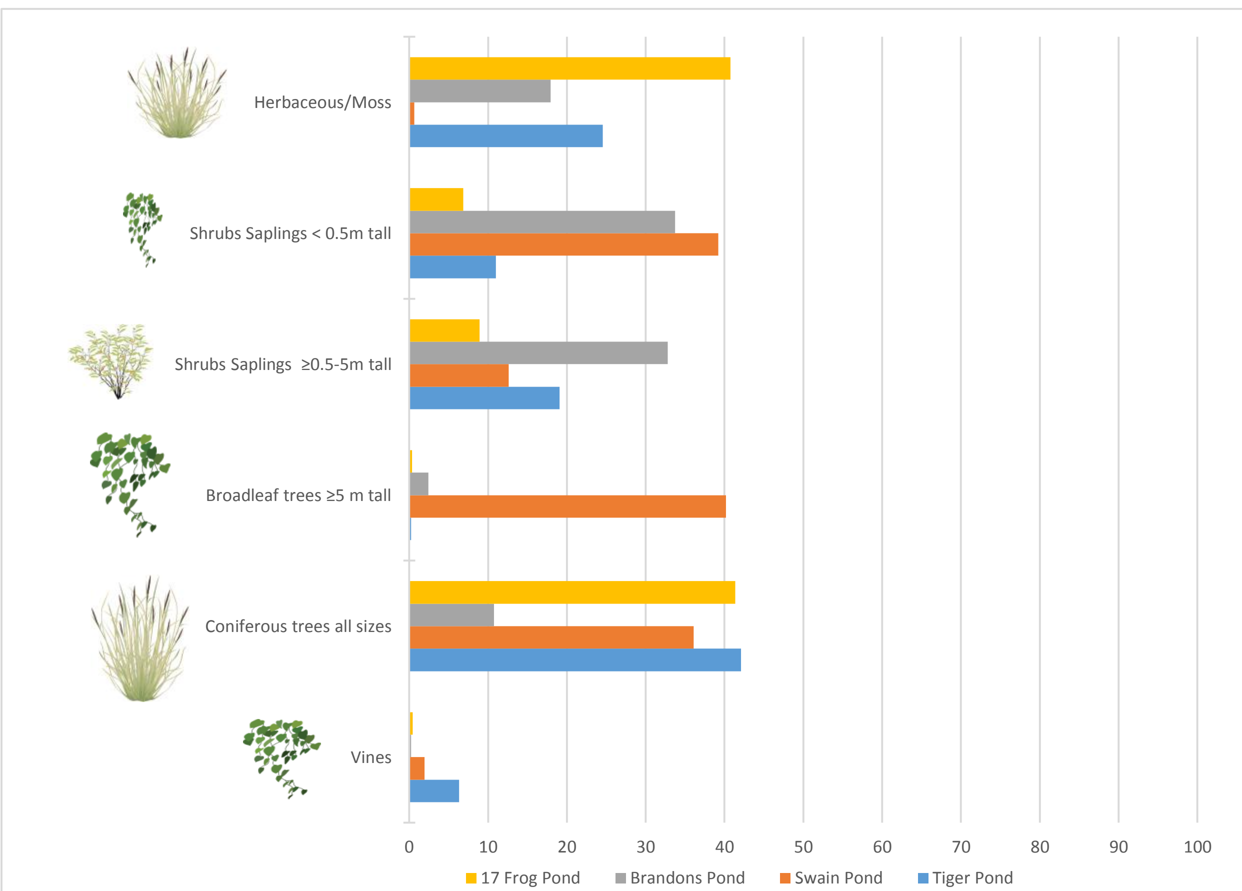


Figure 52. Upland buffer vegetation structure characterization in open canopy reference averaged across year 1 and year 3.

Bars represent mean percent cover in buffer plots.



Macroinvertebrates

Ninety-seven (97) macroinvertebrate taxa were collected, for a total of 2,172 individuals (Table 23). Dipterans made up the majority (35 to 55% of total taxa) of the macroinvertebrates on the open canopy reference sites, followed by Coleopterans and Odonates (Figure 53). We found no mollusks or mayflies in either sampling year on these sites. “Other taxa” included mainly Hymenopterans and Arachnids. Worms were mainly aquatic types. Caddisflies (Trichoptera) were present, but they represented less than 2% of the total taxa.

Figure 53. General taxonomic composition of macroinvertebrates on open canopy reference sites.

Standard error bars are shown.

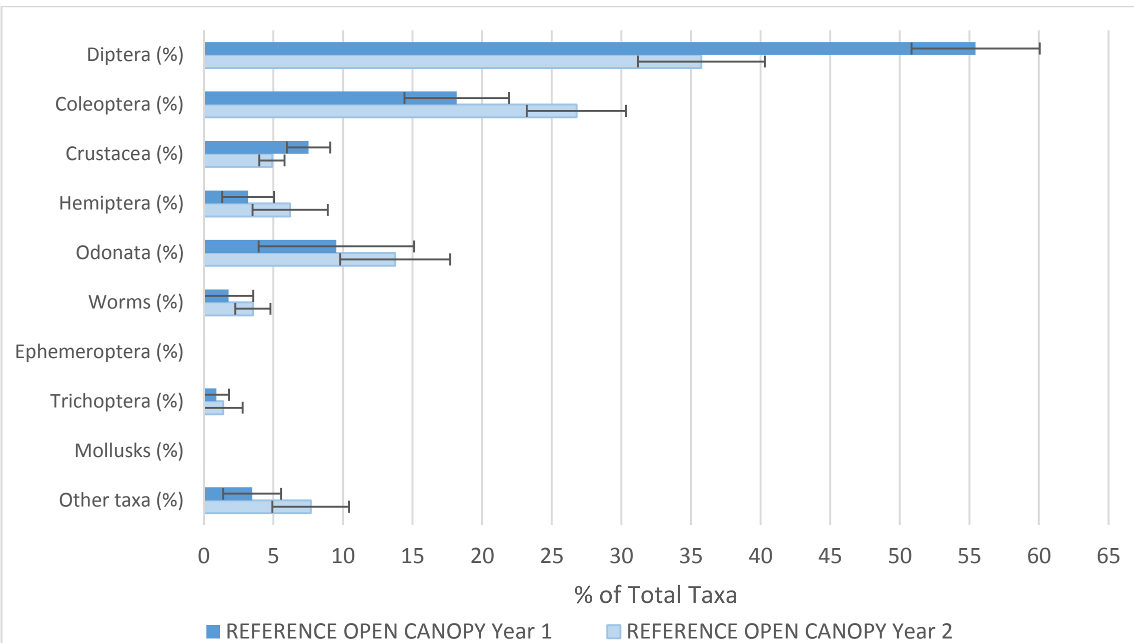


Table 23. Macroinvertebrate taxa found on open canopy reference sites using sweep and bucket samples, years 1 and 2 combined.

Order - Family - Species	Frequency	Abundance	Taxon	Frequency	Abundance
Arachnida			Chironomidae (cont'd)		
Araneae	4	8	Psectrocladius octumaculatus	1	1
Coleoptera			Psectrocladius pilosus	3	178
Curculionidae			Pseudochironomus spp	2	19
Curculionidae (unid)	3	5	Pseudosmittia spp	3	3
Weevil	1	1	Smittia spp	3	5
Dytiscidae			Tanytarsus sp 6	2	8
Agabus spp	2	2	Tvetenia bavarica gr	1	1
Copelatus spp	2	2	Culicidae		
Dytiscidae (unid)	1	1	Aedes spp	1	4
Hydroporus spp	7	34	Anopheles spp	1	1
Ilybius spp	1	2	Culicidae (unid)	1	2
Laccornis spp	3	3	Psorophora spp	1	5
Liodessus spp	1	1	Dolichopodidae	8	47
Neoporus spp	3	17	Limoniidae		
Rhantus spp	2	2	Ormosia spp	1	6
Erirhinidae			Midge (unid, pupae)	2	7
Lissorhoptrus spp	1	26	Tabanidae		
Helophoridae			Chrysops spp	4	6
Helophorus spp	1	1	Terrestrial white maggot	1	1
Hydraenidae			Tipulidae		
Hydraena spp	1	1	Hexatoma spp	1	1
Hydrochidae			Rhabdomastix spp	1	1
Hydrochus sp 6	1	37	**Hemiptera**		
Hydrochus sp 7	1	24	Corixidae		
Hydrophilidae			Sigara spp	3	19
Berosus spp	5	11	Naucoridae		
Enochrus spp	2	3	Pelocoris spp	2	2
Tropisternus blatchleyi	1	1	Nepidae		
Tropisternus collaris	1	1	Ranatra spp	1	1
Tropisternus spp	2	2	Notonectidae		
Terrestrial beetle	1	1	Buenoa spp	3	15
Unidentified beetle fly	1	7	Notonecta spp	2	7
Crustacea			**Hymenoptera (ant)**	3	3
Diplostraca			**Insecta**		
Daphniidae			Mentodea		
Daphnia spp	2	3	Mantis (unid)	1	1
Isopoda			**Lepidoptera (unid)**	1	1
Asellidae			**Odonata**		
Asellus spp	1	1	Aeshnidae		
Ostracoda	8	545	Anax longipes	3	5
Subclass Copepoda (unid)	1	1	Coenagrionidae		
Diptera			Argia spp	1	1
Ceratopogonidae			Enallagma spp	4	93
Bezzia/Palpomyia complex spp	5	18	Ischnura spp	4	110
Dasyhelea spp	1	1	Lestidae		
Chaoboridae			Lestes spp	2	6
Chaoborus punctipennis	2	4	Libellulidae		
Chironomidae			Erythemis simplicicollis	1	14
Ablabesmyia janta	1	4	Erythrodiplax connata	1	1

Order - Family - Species	Frequency	Abundance	Taxon	Frequency	Abundance
Ablabesmyia mallochi	7	195	Libellula spp	5	18
Camptocladius spp	1	2	Libellulidae (unid)	1	1
Chironomini genus III	2	134	Pachydiplax longipennis	3	81
Chironomus spp	6	38	Tramea onusta	3	20
Conchapelopia Hudson sp.	1	6	**Trichoptera**		
Conchapelopia spp	2	5	Hydroptilidae		
Dicrotendipes modestus	1	2	Hydroptilid caddisfly	1	1
Gymnometriocnemus spp	1	1	Leucotrichia spp	1	1
Labrundinia pilosella	1	12	Oxyethira spp	1	4
Larsia spp	1	11	**Worms**		
Limnophyes spp	5	10	**Tubificida**		
Macropelopia spp	1	5	Naididae		
Mesosmittia spp	1	1	Nais spp	1	3
Natarsia spp	1	6	Pristina spp	1	1
Polypedilum illinoense gr	10	164	Tubificidae no hair	1	1
Polypedilum trigonum	1	1	**Lumbriculida**		
Polypedilum tritum	2	6	Lumbriculidae	1	1
Procladius spp	4	17	**Enchytraeida**		
Psectrocladius (Monopsectrocladius) spp	7	77	Enchytraeidae	1	1
Psectrocladius flavus	1	1			

Amphibians

On open canopy reference sites, 19 different amphibian species were detected (13 in 2013, 16 in 2014, and 11 in 2015) (Table 24). Most were anurans (17 species), and the other two species were a newt and a salamander. Only six amphibian species were detected all three years of the study on open canopy reference sites; the majority of species were detected either one or two of the study years. Four species were detected only one of the three years of the study: the Eastern tiger salamander (*Ambystoma tigrinum*), Cope's gray treefrog (*Hyla chrysoscelis*), the ornate chorus frog (*Pseudacris ornate*), and the carpenter frog (*Rana virgatipes*).

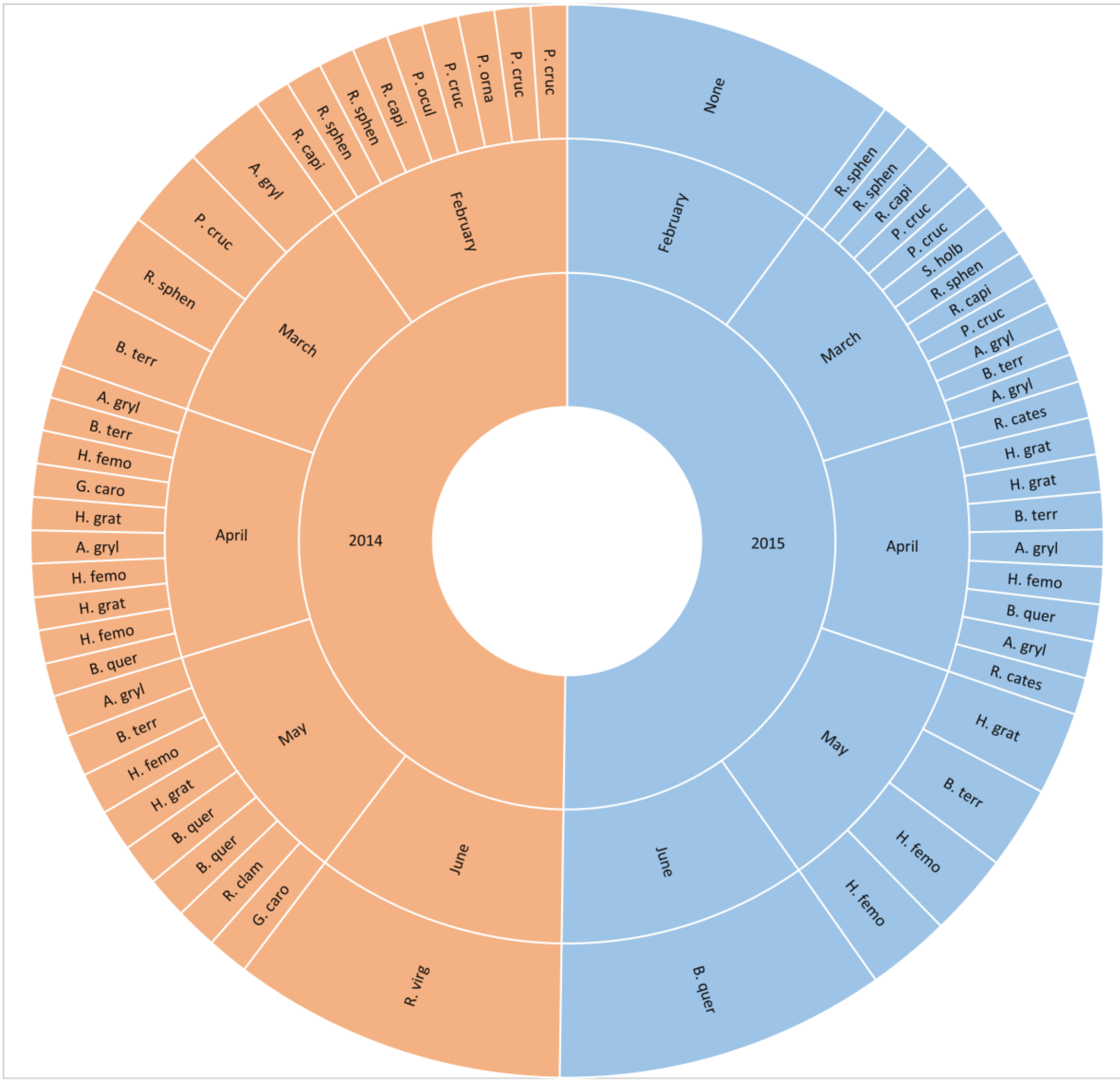
Anuran calling began earlier in 2014 than 2015 (frogloggers were installed to late in the season in 2013 to get first calling data) (Figure 54). Three species in the *Pseudacris* genus were first to call in 2014, followed by the Carolina gopher frog (*Rana capito*) and Southern leopard frog (*R. sphenocephala*). In 2015, a large variety of Ranids (*Rana* sp.), chorus frogs (*Pseudacris* sp.) toads (*Scaphiopus* sp. and *Bufo* sp.), and cricket frogs (*Acris gryllus*) all began calling in March. In 2014, the carpenter frog (*Rana virgatipes*) was the last species to initiate calling, while in 2015, the oak toad (*Bufo quercicus*) was the last species to initiate calling.

Table 24. Amphibian species detected on open canopy reference sites using observations with dipnets and egg mass surveys (O), and acoustic recordings with a froglogger (F).

Common Name	Species Name	2013	2014	2015	Total Years
Southern Cricket Frog	*Acris gryllus*	OF	OF	OF	3
Eastern Tiger Salamander	*Ambystoma tigrinum*		O		1
Oak Toad	*Bufo quercicus*	F	F	F	3
Southern Toad	*Bufo terrestris*	F	F	F	3
Eastern Narrowmouth Toad	*Gastrophryne carolinensis*	OF	F		2
Cope's Gray Treefrog	*Hyla chrysoscelis*	F			1
Pinewoods Treefrog	*Hyla femoralis*	OF	OF	F	3
Barking Treefrog	*Hyla gratiosa*	OF	OF	OF	3
Squirrel Treefog	*Hyla squirella*	F	O		2
Eastern Newt	*Notophthalamus viridescens*		O	O	2
Spring Peeper	*Pseudacris crucifer*		OF	F	2
Little Grass Frog	*Pseudacris ocularis*	F	F		2
Ornate Chorus Frog	*Pseudacris ornata*		F		1
Carolina Gopher Frog	*Rana capito*		OF	F	2
American Bullfrog	*Rana catesbeiana*	F		F	2
Green Frog	*Rana clamitans*	F	F		2
Southern Leopard Frog	*Rana sphenocephala*	OF	OF	OF	3
Carpenter Frog	*Rana virgatipes*		F		1
Eastern Spadefoot	*Scaphiopus holbrookii*	OF		F	2
Unidentified (egg mass)	Unidentified (egg mass)	O			1

Figure 54. Months of first detection of calling species by frogloggers in the four closed canopy reference wetlands for 2014 and 2015.

Multiple mention of a species indicates separate sites within each year. Species are identified using the first letter of genus and first four letters of species name. Names appear in order of detection within a given year.



PROFILE OF ENHANCEMENT WETLANDS

Landscape Attributes

Generally, development intensity within the 500-meter buffer of the enhancement wetlands was very low, nearly completely undisturbed by anthropogenic land use changes (Table 25). Development intensity within one mile of the enhancement wetlands varied from 1.5 (modified natural area) to 3.1 (levels similar to pastureland, pine plantations, and recently logged areas).

The 500-meter buffers of the enhancement wetland sites were primarily natural land, along with herbaceous areas, primarily feed lots (Figure 55). The 500-meter buffers around the enhancement wetlands were almost completely vegetated.

The 1-mile buffer surrounding the enhancement wetlands was nearly 65% natural land, over 85% vegetated (natural + planted), and approximately 14% impacted (clearcut, developed, or barren) (Figure 56). The 1-mile area surrounding the enhancement wetlands included more significant pine plantations, and included a small amount of severely impacted areas (high density development and industrial).

Table 25. Landscape Development Intensity Index (LDI) values for enhancement wetland site buffers.

Site Name	County	LDI - 500 m	LDI - 1 mile
Block T Pond	Richmond	1.1	3.1
Braswell Ponds	Carteret	1.1	1.5
Little Little Dismal Pond	Richmond	1.1	2.0
Slate Circle Pond	Scotland	2.3	1.7
Mean LDI Value		1.4	2.1

Figure 55. Distribution of land cover types within 500-meter buffers of enhancement wetlands.

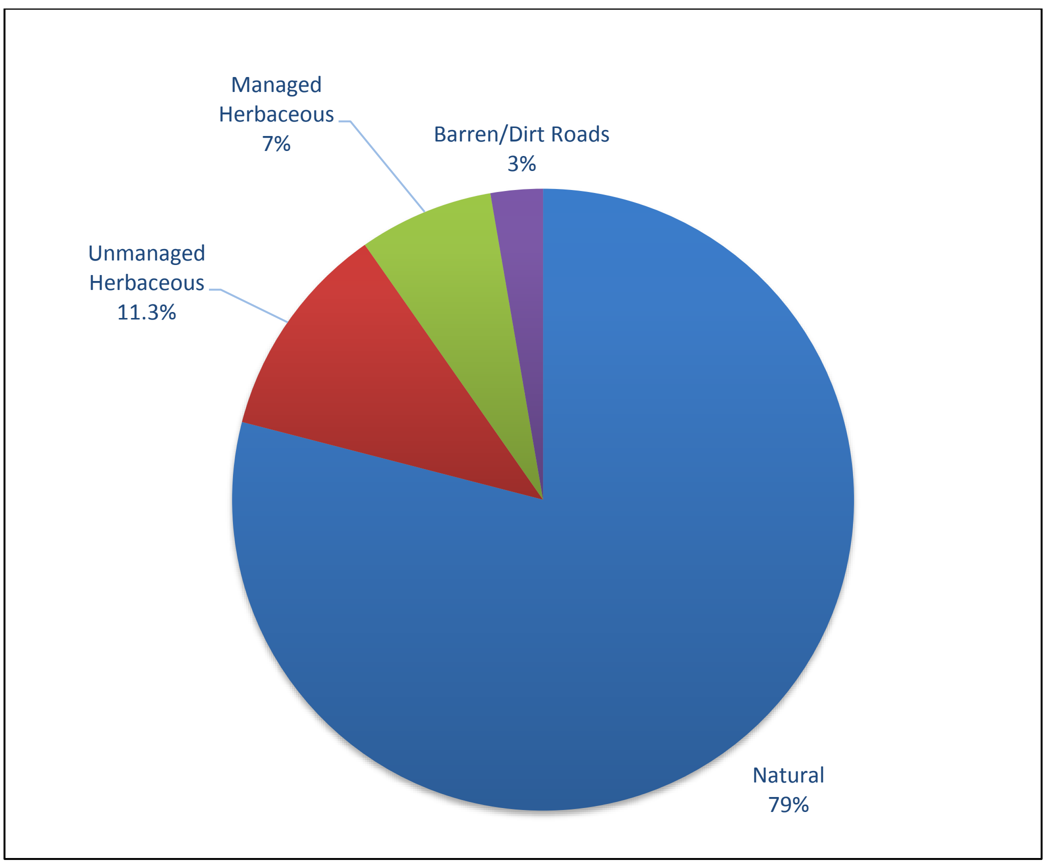
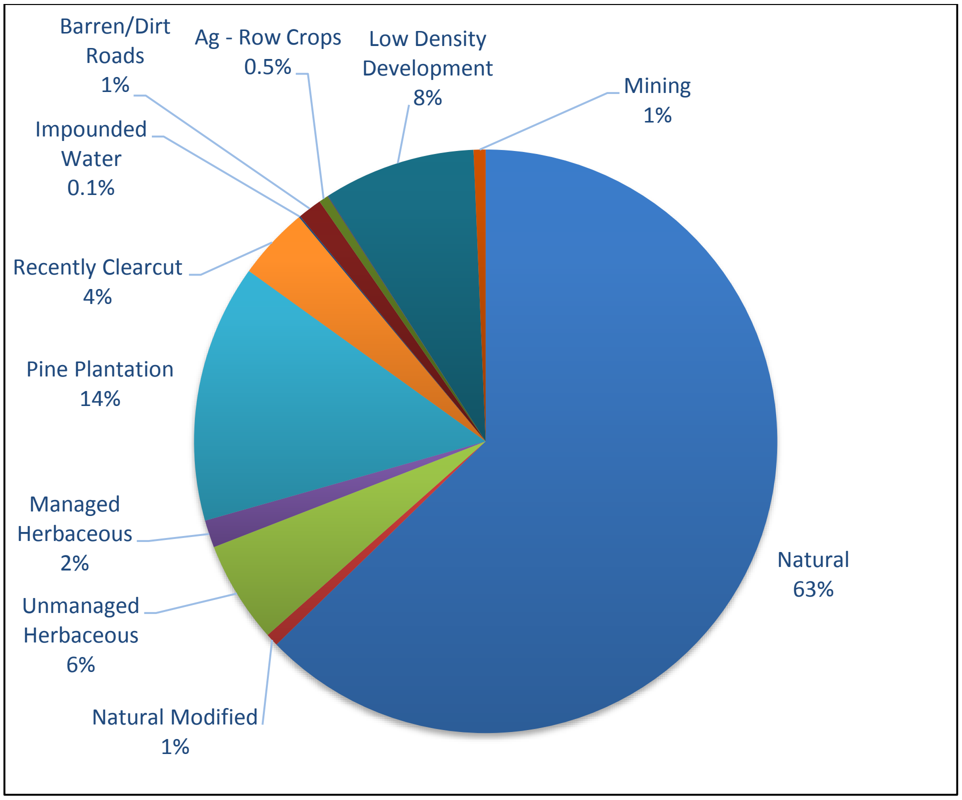


Figure 56. Distribution of land cover types within 1-mile buffers of enhancement wetlands.



Rapid Assessments

All enhancement sites were nearly all rated high quality by the NCWAM, and ranged from minimal to superior habitat quality by the ORAM (Table 26). They varied in total wetland size from 0.1 acre to just over 12 acres.

Table 26. General information and rapid assessment results for enhancement sites.

NC WRC = NC Wildlife Resources Commission; USFWS = United States Fish and Wildlife Service; NHP = NC Natural Heritage Program; NCWAM = NC Wetland Assessment Method; ORAM = Ohio Rapid Assessment.

Site Name	County	Total Size (acres)	Ownership	NCWAM Type	NHP Type	NCWAM	ORAM Rating	ORAM Score (out of 90)
Block T Pond	Richmond	12.06	NC WRC	Basin	Small Depression Pond	High	Moderate	61.5
Braswell Ponds	Carteret	0.097	USFWS	Basin	Small Depression Pond	High	Moderate	50.5
Little Little Dismal	Richmond	0.5	NC WRC	Basin	Upland Pool	High	Superior	65.5
Slate Circle	Scotland	0.89	NC WRC	Basin	Vernal Pool	Low	Minimal	41

Water Quality

The highest mean pH of all sites is this study was on a restoration site (Slate Circle) (Table 27). Mean pH on these sites ranged from 3.4 to 5.9. Mean dissolved oxygen was highly variable by site and by year.

Table 27. Mean field water quality parameters by year on enhancement sites.

(Meter malfunctioned in 2015 at Block T Pond).

	Mean pH			Mean Specific Conductivity (µS/cm)			WQ mean Dissolved Oxygen (mg/mL)		
Year	2013	2014	2015	2013	2014	2015	2013	2014	2015
Block T Pond	4.28	4.14	4.26	46.9	33.7	28.6	6.25	7.78	n/a
Braswell Ponds	3.73	3.68	4.13	45.7	37.4	31.7	5.65	10.48	2.88
Little Little Dismal Pond	3.54	3.40	3.77	46.2	28.8	37.0	4.70	8.70	9.81
Slate Circle	5.30	5.31	5.93	43.4	34.0	37.2	4.20	7.50	10.62

Hydrology

Water depth at sampling points on the enhancement sites varied between nearly 2 feet deep to no water at all (Figure 57). Block T site contained much more water in the summer of 2013 than in 2014 and 2015 (Figure 58). All sites went dry (except Braswell Ponds) at some point during the study. Three of the four sites appeared to be sensitive to precipitation events, while one (Braswell Ponds) did not (Figures 59-62). All sites (except Braswell Ponds) showed a seasonal hydrology cycle, with high water levels in the late winter/spring and low or dry levels in late summer.

Figure 57. Mean water depth at sampling points within enhancement sites over time.

Braswell Pond site was not able to be visited March 2015.

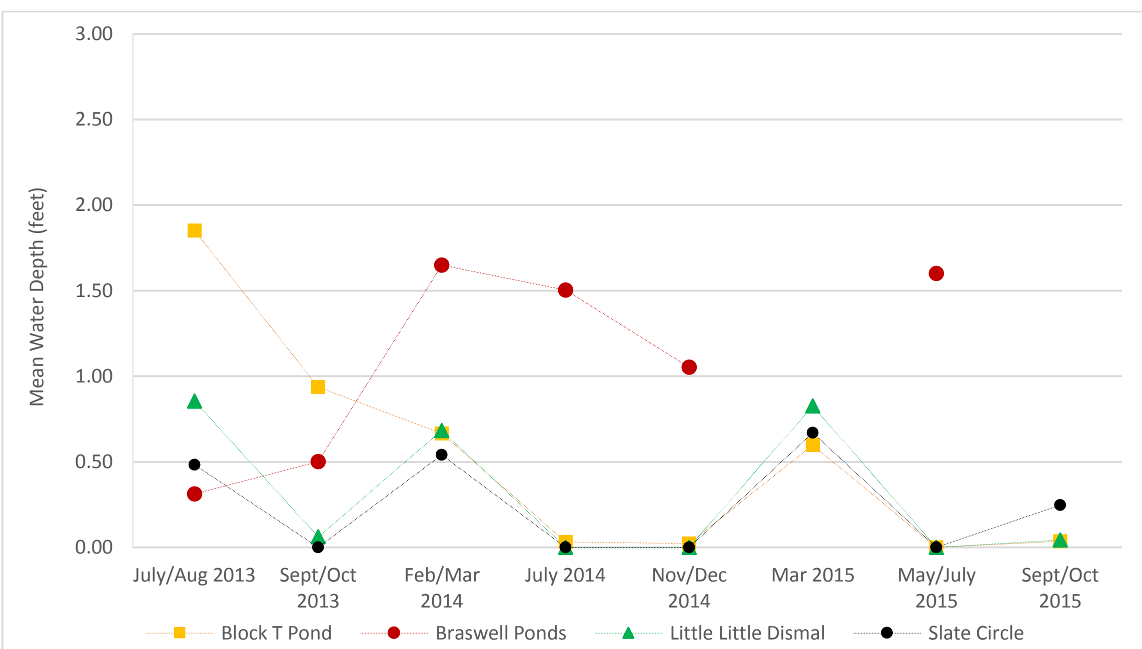


Figure 58. Pool volume for enhancement sites over time.

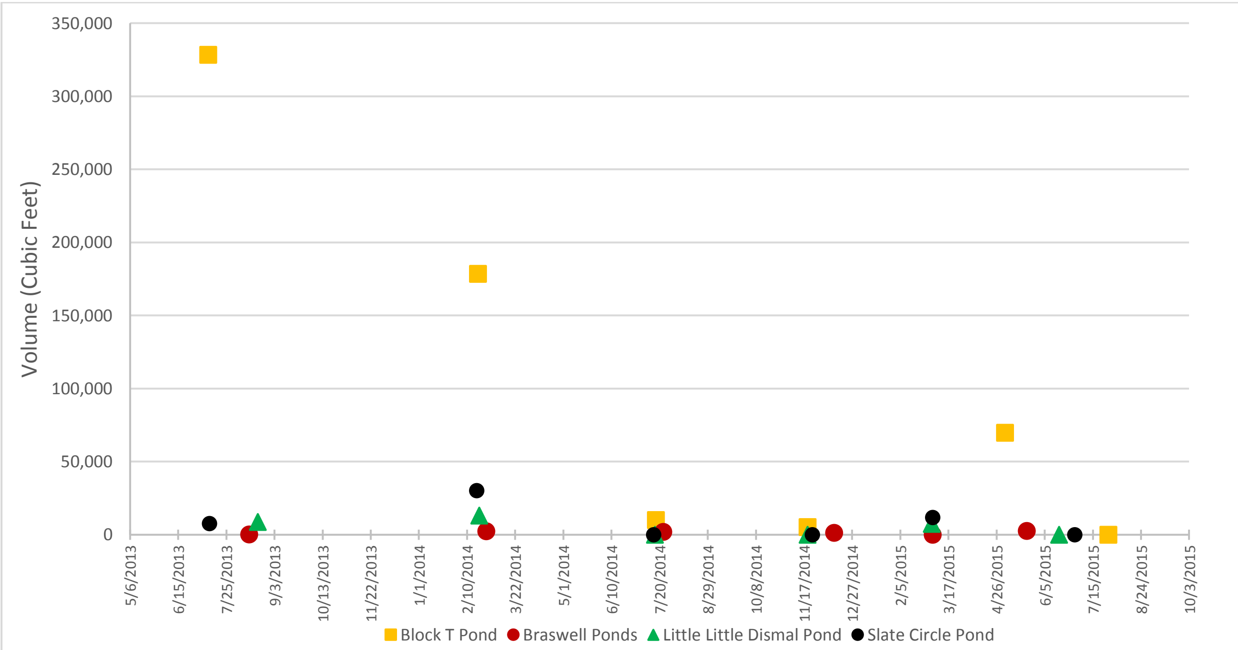


Figure 59. Hydrograph and precipitation data for Block T Pond.

The well at this site was at ground surface level, so it was only able to detect above ground water levels. Water was 1.93 ft below surface on July 28, 2015 (hand dug pit during vegetation survey).

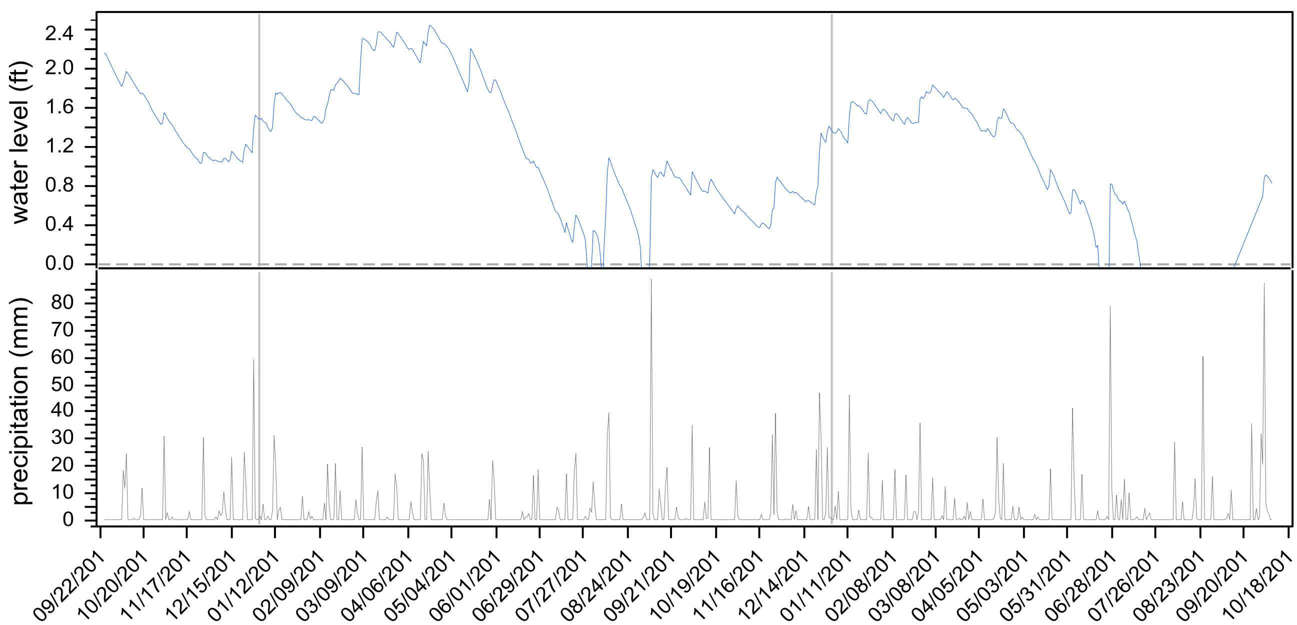
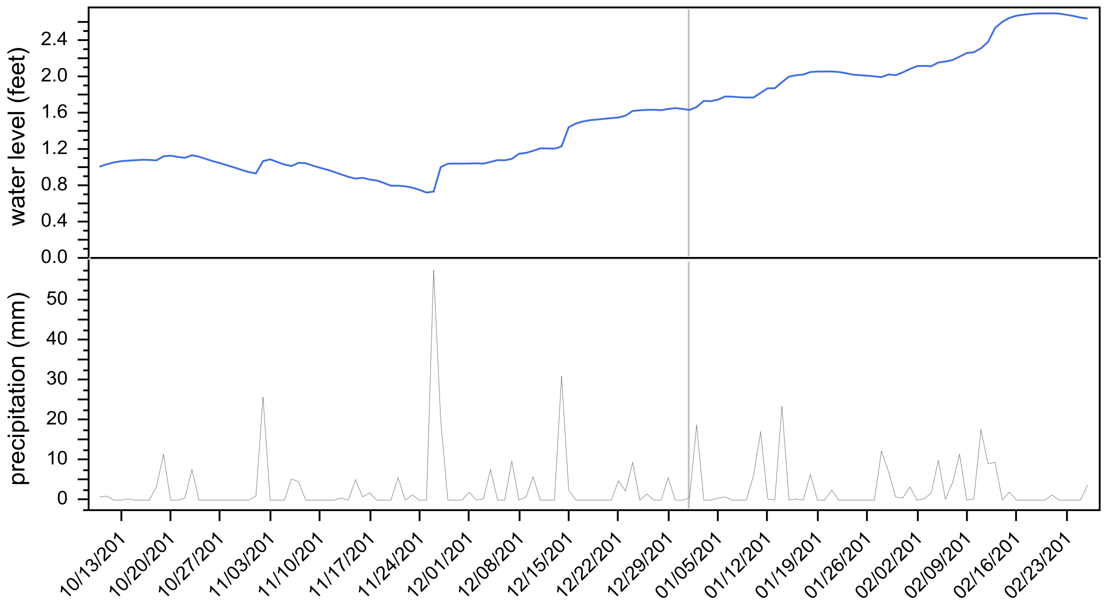


Figure 60. Hydrograph and precipitation data for Braswell Ponds.

Well transducer flooded after February 26, 2014 and could not be replaced until May 2015. Further data were obtained for May 2015 through August 2016 (second graph). Water was always above ground surface level during the recorded period.



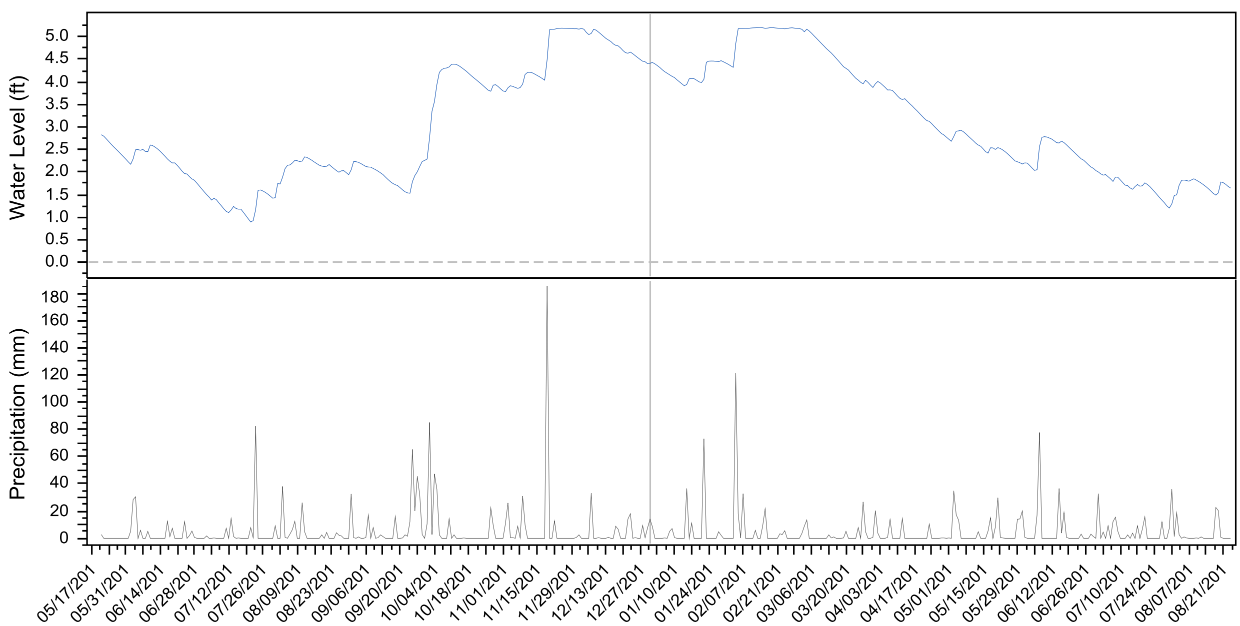


Figure 61. Hydrograph and precipitation data for Little Little Dismal Pond.

Well was unable to sense water levels lower than 1.7 feet below surface. Dotted line represents ground surface level.

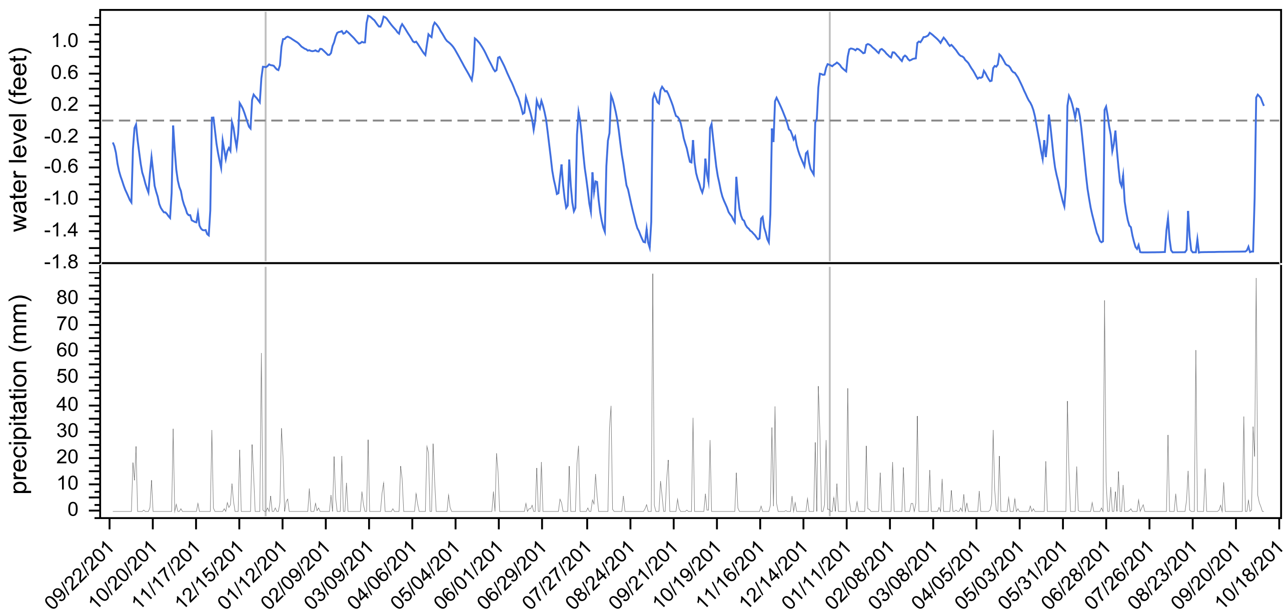
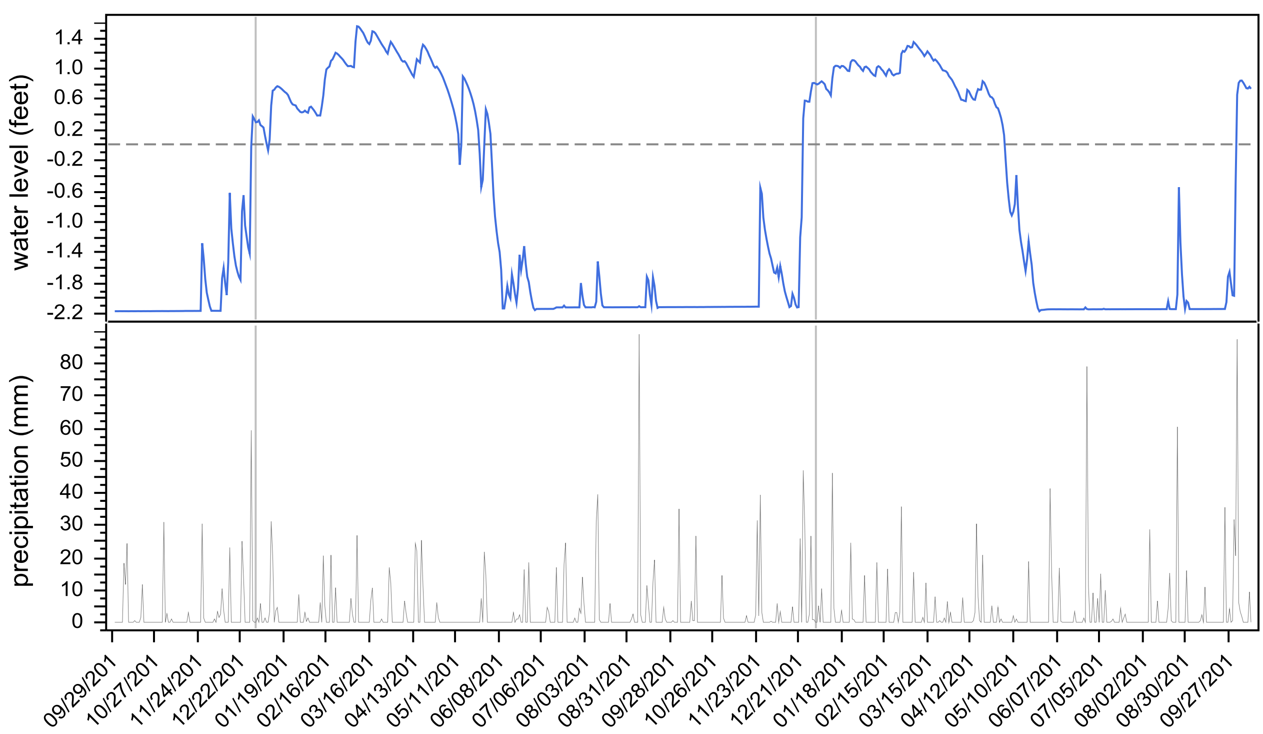


Figure 62. Hydrograph and precipitation data for Slate Circle.

Well was unable to sense water levels lower than 2.2 feet below surface. Dotted line represents ground surface level.



Vegetation

Enhancement sites were dominated by grass and sedge species with usually one tree species present (rapid recolonizers like red maple and sweetgum) (Table 28). Some forbs were also present. The vegetation structure on the enhancement wetlands was consistently dominated by lower size classes (<2m tall), resulting in an environment similar to the open canopy reference wetlands (Figure 63). Tall trees (>5m tall) were not present on the enhancement sites.

Greater herbaceous cover was recorded in the upland buffers around the enhancement sites than at other site types. In general, all cover types were represented in the buffers, except large broadleaf trees which were mainly present at Block T (Figure 64). Vine cover was minimal in the enhancement site buffers, except on Slate Circle which was burned in a controlled fire between sampling years. Block T and Little Little Dismal buffers were also burned between sampling years. Braswell Ponds site had not been burned in recent history (at least 10 years).

Table 28. Dominant plant species in enhancement reference wetlands.

Site	Scientific Name	Common Name	Growth Form
Block T Pond	*Dichanthelium acuminatum*	tapered rosette grass	herbaceous
	Liquidambar styraciflua	sweetgum	tree
	Panicum hemitomon	maidencane	herbaceous
	Sacciolepis striata	American cupscale	herbaceous
Braswell Ponds	*Arundinaria tecta*	switchcane	herbaceous
	Carex gigantea	giant sedge	herbaceous
	Cyrilla racemiflora	swamp titi	shrub
	Liquidambar styraciflua	sweetgum	tree
	Rhynchospora macrostachya	tall horned beaksedge	herbaceous
Little Little Dismal	*Acer rubrum*	red maple	tree
	Carex sp.	sedge	herbaceous
	Dichanthelium sp.	rosette grass	herbaceous
	Dulichium arundinaceum	threeway sedge	herbaceous
	Lyonia ligustrina	maleberry	shrub
	Scirpus cyperinus	woolgrass	herbaceous
	Sphagnum sp.	sphagnum	moss
	Zenobia pulverulenta	honeycup	herbaceous
Slate Circle	*Andropogon glaucopsis*	purple bluestem	herbaceous
	Apocynum cannabinum	Indianhemp	herbaceous
	Chamaecrista sp.	partridge pea	herbaceous
	Dichanthelium acuminatum	tapered rosette grass	herbaceous
	Diodia virginiana	Virginia buttonweed	herbaceous
	Lespedeza cuneata	Chinese bush-clover	herbaceous
	low green grass, unidentified	grass	herbaceous
	Poaceae	grass	herbaceous
	Rubus cuneifolius	sand blackberry	shrub
	Solidago altissima	Canada goldenrod	herbaceous
	Solidago sp.	goldenrod	herbaceous

Figure 63. Wetland vegetation structure characterization in enhancement wetlands averaged across years 1 and 3.

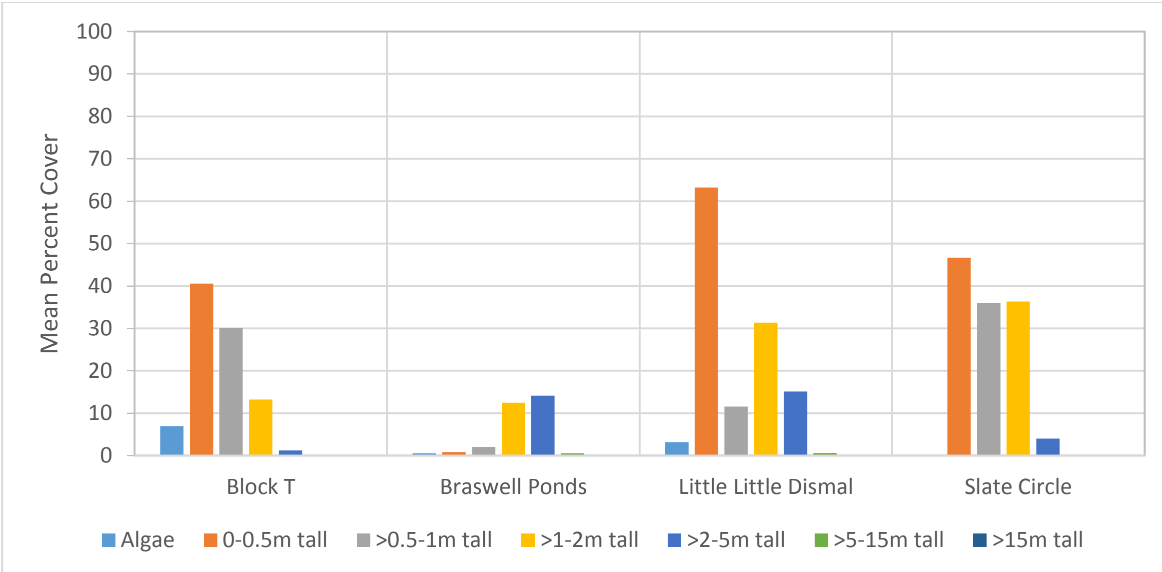
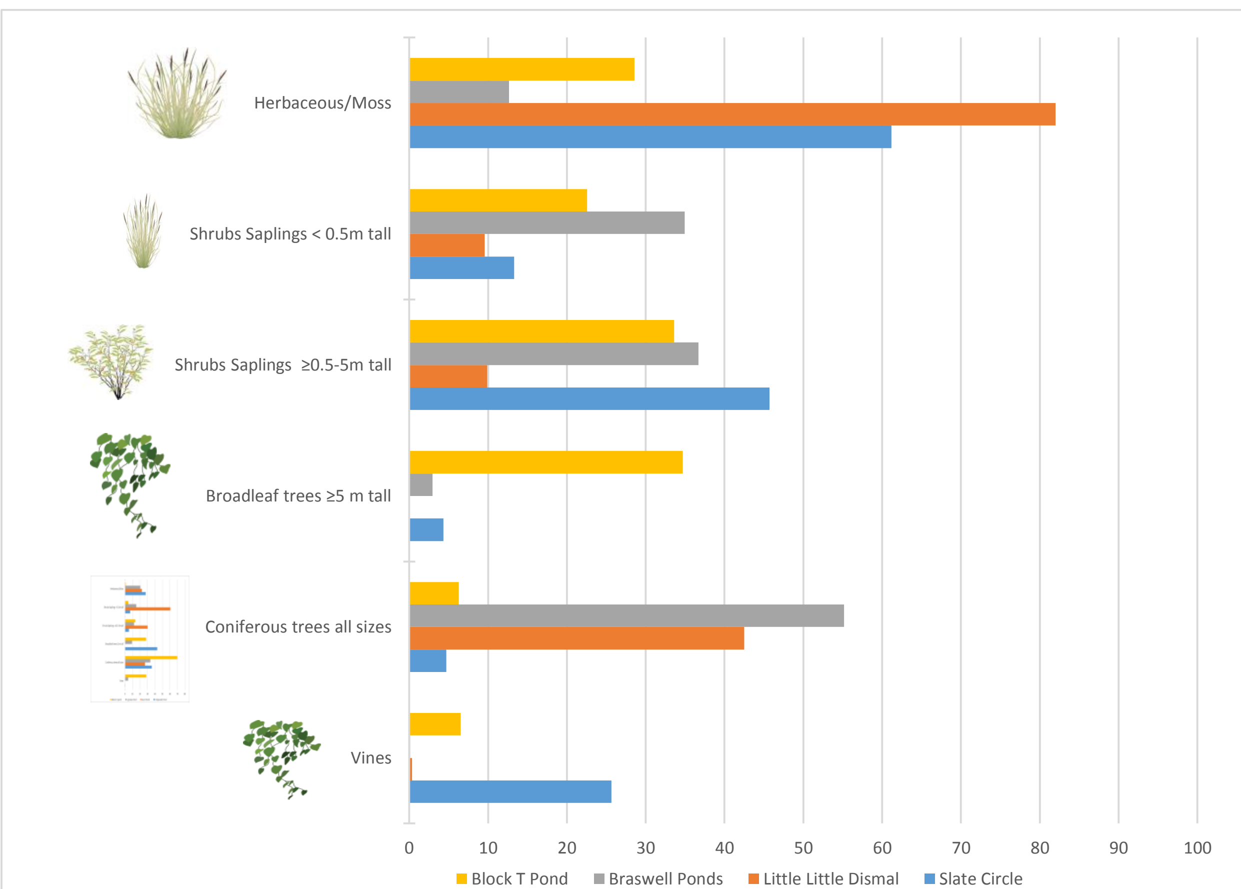


Figure 64. Upland buffer vegetation structure characterization in enhancement averaged across year 1 and year 3.

Bars represent mean percent cover in buffer plots.



Macroinvertebrates

One hundred one (101) macroinvertebrate taxa were identified on enhancement sites, including a total of 5,671 individuals (Table 29). Dipterans made up the majority (45-50% of total taxa) of the macroinvertebrate taxa on the enhancement sites, followed by coleopterans, crustaceans, and worms (Figure 65). Worms included aquatic and terrestrial types. Enhancement sites also contained one mollusk taxon in the second sampling year, a group missing from all reference sites. This mollusk was a freshwater limpet (*Ferrissia* sp.). "Other taxa" included mainly Arachnids and Springtails (Class Collembola). The mayflies (Ephemeroptera) and caddisflies (Trichoptera) were all present on the enhancement sites, but together they represented less than 3% of the total taxa.

Odonates, Hemipterans, Trichopterans, and Mollusk taxa increased substantially between the first year and the second on the enhancement sites.

Figure 65. General taxonomic composition of macroinvertebrates on enhancement sites.

Standard error bars are shown.

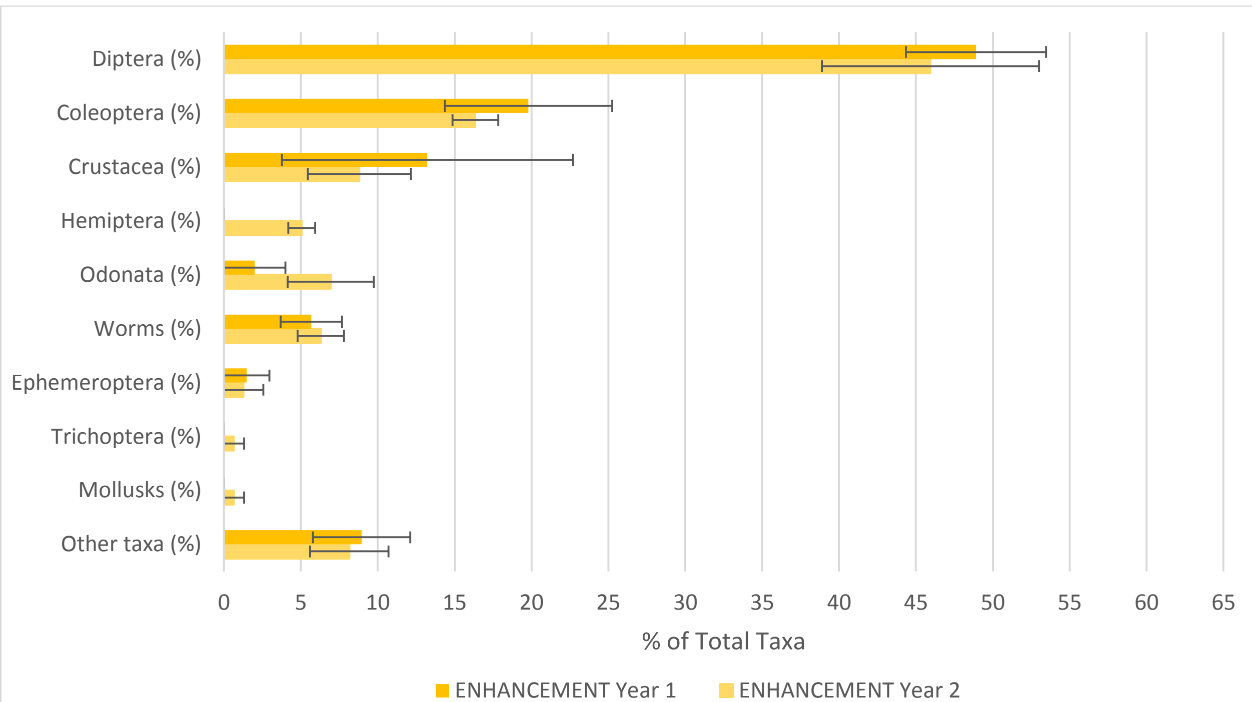


Table 29. Macroinvertebrate taxa (identified to the lowest taxonomic level) found on enhancement sites using sweep and bucket samples, years 1 and 2 combined.

Order - Family - Species	Freq.	Abund.	Taxon	Freq.	Abund.
Arachnida			Chironomidae (cont'd)		
Araneae	5	18	Paratanytarsus spp	1	1
Class Collembola			Polypedilum illinoense gr	10	779
Order Collembola	5	10	Polypedilum tritum	4	51
Coleoptera			Procladius spp	4	55
Carabidae	1	2	Psectrocladius (Monopsectrocladius) spp	3	1154
Curculionidae	2	4	Psectrocladius flavus	1	1
Dytiscidae			Psectrocladius pilosus	2	10
Agabus spp	3	3	Psectrocladius spp	1	2
Copelatus spp	1	3	Pseudochironomus spp	1	1
Dytiscus spp	1	1	Pseudosmittia spp	3	18
Hydroporus spp	5	16	Smittia spp	3	9
Ilybius spp	2	9	Tanytarsus sp G	1	27
Laccophilus proximus	2	4	Culicidae		
Laccophilus spp	1	1	Aedes spp	1	2
Laccornis spp	1	1	Anopheles spp	1	1
Liodessus crothi	1	1	Culiseta spp	1	15
Liodessus spp	3	37	Mosquitoe (unid)	2	17
Neoporus spp	2	4	Psorophora spp	1	9
Rhantus spp	1	1	Dolichopodidae	7	18
Helophoridae			Limoniidae		
Helophorus spp	2	6	Antocha spp	1	1
Hydrochidae			Ormosia spp	3	6
Hydrochus spp	2	3	Terrestrial white maggot	3	12
Hydrophilidae			Tipulidae		
Berosus spp	5	15	Tipula spp	1	1
Enochrus spp	2	2	**Ephemeroptera**		
Staphylinidae	1	1	Baetidae		
Crustacea			Callibaetis spp	1	23
Amphipoda			Centroptilum spp	1	6
Crangonyctidae			Caenidae		
Crangonyx serratus	4	47	Caenis spp	1	5
Copepoda			**Hemiptera**		
Copepod, unidentified	4	12	Cicadellidae	2	4
Decapoda			Corixidae		
Astacidae	1	1	Sigara spp	1	1
Cambaridae			Notonectidae		
Procambarus spp	2	9	Notonecta spp	3	4
Isopoda			**Hymenoptera**	1	1
Asellidae			**Insecta**		
Asellus spp	1	208	**Blattodea**		
Caecidotea attenuata	1	64	Termite	1	4
Caecidotea obtulus	1	286	**Lepidoptera**	1	1
Caecidotea spp	1	175	**Mollusk**		
Ostracoda			**Basommatophora**		
Seed shrimp			Ancylidae		
Ostracod	7	1045	Ferrissia spp	1	4
Diptera			**Odonata**		
Ceratopogonidae			Coenagrionidae		
Bezzia/Palpomyia complex spp	3	26	Enallagma spp	2	8
Dasyhelea spp	2	8	Ischnura spp	1	2
Palpomyia spp	2	6	Lestidae		
Chaoboridae			Lestes spp	2	10

Order - Family - Species	Freq.	Abund.	Taxon	Freq.	Abund.
Chaoborus punctipennis	1	2	Libellulidae		
Mochlonyx spp	2	5	Celithemis spp	1	1
Chironomidae			Erythemis simplicicollis	1	1
Ablabesmyia aspera	1	11	Libellula spp	1	2
Ablabesmyia illinoense	1	50	Pachydiplax longipennis	2	6
Ablabesmyia mallochi	2	32	**Trichoptera**		
Ablabesmyia parajanta group	1	5	Hydroptilidae		
Ablabesmyia rhamphe gr	1	16	Oxyethira spp	1	2
Camptocladius spp	1	6	**Worms**		
Chaetocladius spp	2	9	**Phylum Annelida**		
Chironomus spp	6	119	Achaetous worm	1	8
Conchapelopia spp	1	1	**Superorder Megadrilacea**		
Dicrotendipes modestus	2	257	Megadrile	2	47
Dicrotendipes nervosus	2	24	**Tubificida**		
Guttipelopia guttipennis	1	105	Naididae		
Gymnometriocnemus spp	1	1	Dero spp	1	4
Kiefferulus dux	1	1	Nais spp	1	1
Kiefferulus spp	4	222	Tubificidae no hair	1	1
Larsia spp	1	7	**Lumbriculida**		
Limnophyes spp	6	18	Lumbriculidae	4	28
Natarsia spp	3	382	**Enchytraeida**		
Parachironomus carinatus	1	4	Enchytraeidae	1	1

Amphibians

On enhancement sites, 19 different amphibian species were detected (16 in 2013, 15 in 2014, and 15 in 2015) (Table 30). Most were anurans (17 species), and the other two species were a newt and a salamander. Approximately half of the amphibian species (10 of 19) were detected all three years of the study on re-establishment sites. The Eastern tiger salamander (*Ambystoma tigrinum*) and the pine barrens treefrog (*Hyla andersonii*) were the only species detected during only one year; all other species were detected at least two years of the study.

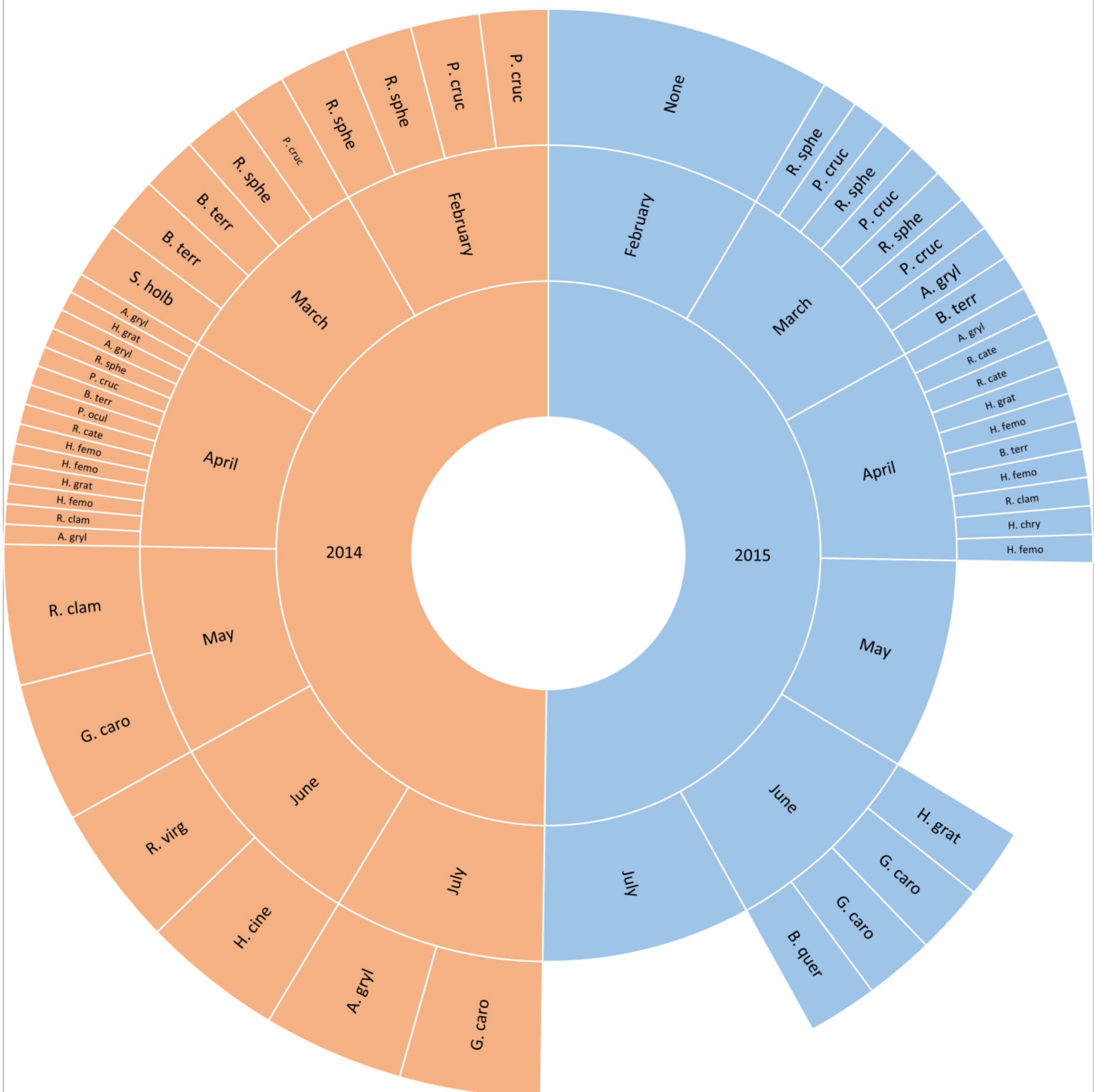
Anuran calling began earlier in 2014 than 2015 (frogloggers were installed to late in the season in 2013 to get first calling data) (Figure 66). Spring peepers (*Pseudacris crucifer*) and Southern leopard frogs (*Rana sphenocephala*) were the first species to call at enhancement sites in both years, as is typical for these species. They were followed by the southern toad (*Bufo terrestris*). In 2014, the Eastern narrowmouth toad (*G. carolinensis*) was the last species to initiate calling, while in 2015, the Eastern narrowmouth toad and oak toad (*Bufo quercicus*) were the last species to initiate calling (the oak toad never called in 2014, but did call in 2013).

Table 30. Amphibian species detected on enhancement sites using observations with dipnets and egg mass surveys (O), and acoustic recordings with a froglogger (F).

Common Name	Species Name	2013	2014	2015	Total Years
Southern Cricket Frog	*Acris gryllus*	OF	OF	OF	3
Eastern Tiger Salamander	*Ambystoma tigrinum*		O		1
Oak Toad	*Bufo quercicus*	F		F	2
Southern Toad	*Bufo terrestris*	OF	OF	F	3
Eastern Narrowmouth Toad	*Gastrophryne carolinensis*	OF	F	OF	3
Pine Barrens Treefrog	*Hyla andersonii*	OF			1
Cope's Gray Treefrog	*Hyla chrysoscelis*	OF		F	2
Green Treefrog	*Hyla cinerea*	F	F		2
Pinewoods Treefrog	*Hyla femoralis*	OF	OF	OF	3
Barking Treefrog	*Hyla gratiosa*	OF	OF	OF	3
Squirrel Treefog	*Hyla squirella*	F		O	2
Eastern Newt	*Notophthalamus viridescens*		O	O	2
Spring Peeper	*Pseudacris crucifer*	F	OF	OF	3
Little Grass Frog	*Pseudacris ocularis*	OF	OF	O	3
American Bullfrog	*Rana catesbeiana*		F	F	2
Green Frog	*Rana clamitans*	OF	OF	F	3
Southern Leopard Frog	*Rana sphenocephala*	OF	OF	OF	3
Carpenter Frog	*Rana virgatipes*	F	F		2
Eastern Spadefoot	*Scaphiopus holbrookii*	OF	F	OF	3

Figure 66. Months of first detection of calling species by frogloggers in the four enhancement wetlands for 2014 and 2015.

Species are identified using the first letter of genus and first four letters of species name. Multiple mention of a species indicates separate sites within each year. Names appear in order of detection within a given year.



PROFILE SUMMARIES

The following is a summary table of the various feature of each site type (Table 31).

Table 31. Feature summaries of each site type.

Site Type Characteristics	Landscape Level/Rapid Assessments	Water	Vegetation Structure/Composition	Aquatic Life
Closed Canopy Reference Sites	- best ORAM scores (habitat quality) of any site type and best NCWAM ratings (function)	- very acidic water condition (median pH = 3.95); narrowest pH range of all site types - variable hydroperiods from 5 months to 12 months	- high amounts of canopy cover (median 60%) - plant structure size distribution skewed toward middle and large size classes: very little herbaceous or <2m tall vegetation; much more cover by broadleaf trees and vines than re-establishment sites - large amounts of large and small woody debris	- mostly fish free - lowest number of macroinvertebrate species of any site type - broad range of AQAI values and amphibian species sizes
Re-establishment Sites	- typically more developed (higher LDI) in 500m buffer and 1 mile surrounding wetland than reference - worse ORAM score (habitat quality) and NCWAM ratings (function) than reference - widest range of distances to nearest natural wetland of all site types	- less acidic than all other site types, but still acidic (median pH = 4.6); lower median dissolved oxygen (34%) than reference (45.5%) - highest specific conductivity of any site type (median of approximately 60 uS/cm) - comparable water temperature to reference sites - permanent water	- less canopy cover (10%) than reference - plants structure size distribution skewed toward small and middle size classes: much higher emergent plant and algae cover than reference; lower cover by trees and small shrubs/saplings than reference - less total litter than reference; very little large dead woody debris, compared to reference - low floristic quality (FQAI) compared to reference	- fish present - highest number of macroinvertebrate taxa and highest diversity of all site types - higher macroinvertebrate density than reference - more tolerant macroinvertebrates - more macroinvertebrate taxonomic groups represented than closed ref; fewer dipterans than other site types; more beetles than reference - slightly higher amphibian richness than reference, but lower species quality (AQAI) than reference - the smallest amphibian species were absent

Site Type Characteristics	Landscape Level/Rapid Assessments	Water	Vegetation Structure/Composition	Aquatic Life
Open Canopy Reference Sites	- more developed within 1 mile than enhancement sites; comparable at 500m - farther from roads and closer to natural wetlands than enhancement sites - better ORAM scores (habitat quality) and NCWAM ratings (function) than enhancement sites	- highest median dissolved oxygen of any site type (90.4%); significantly higher than enhancement sites (median 70.8%) (Wilcoxon test p = 0.015) - the most acidic water of all site types (median pH = 3.75) - warmer water than enhancement sites (median 5 degrees C higher) - highest variability in depth of water of all site types - more standing water than enhancement (most had standing water all year)	- significantly more aquatic vegetation and algae than enhancement sites (Wilcoxon test p<0.05 for both tests) - more low-growing vegetation than enhancement sites	- mostly fish free - ratios of sensitive/tolerant macroinvertebrate species similar to enhancement sites - macroinvertebrate density, abundance, and richness similar to enhancement sites - better amphibian species quality (AQAI) than enhancement sites; comparable richness, and adult species size (but lower abundance)
Enhancement Sites	- less developed within 1 mile than reference sites; comparable at 500m - closer to roads and farther from natural wetlands than reference sites - worse ORAM scores (habitat quality) and NCWAM ratings (function) than reference sites	- lower dissolved oxygen than reference, lower water temperature (median 5 degrees C cooler); specific conductivity similar to reference - generally less acidic than reference (median pH = 4.1); widest pH range of all site types (pH 3.4 to 5.9) - variable hydroperiods from 5 months to 12 months	- more cover of mid-sized plants (1-5m tall) than reference sites (including more vine cover, shrubs and saplings, herbaceous) - more litter than open reference sites; more large woody debris - slightly worse floristic quality than reference (FQAI)	- all fish free - ratios of sensitive/tolerant macroinvertebrates similar to reference - enhancement sites had representation from 2 more macroinvertebrate taxonomic groups than reference (Ephemeroptera and Mollusca) - comparable amphibian richness to reference sites; higher abundance, lower amphibian species quality (AQAI) than reference

One major factor used to guage success of wetland re-establishment sites is the number of days that ground or surface water is present within 12 inches of surface during the growing season. Hydrology success criteria in North Carolina currently require mitigation sites to maintain groundwater measurements to a minimum of ≤12 inches from the surface for 5% to 12.5% (consecutive days) of the growing season. Growing season dates were gathered from information available through the Natural Resources Conservation Service (NRCS 2016), and hydrology well data were used to calculate the percentage of total days when water levels were within 12 inches of surface on consecutive days. Most sites greatly exceeded the minimum required to evaluate success of wetland re-establishment sites (Table 32); data are reported for all sites as comparisons.

Table 32. Percent consecutive days during the growing season with water levels within 12" of surface for all study site wetlands in 2014 and 2015.

Site Type	Site Name	Percent Hydrology Within 12" of Surface		
		2014	2015	Average
Closed Canopy Reference	Block O	29%	17%	23%
	Cypress Pond	100%	100%	100%
	Gum Pond	39%	100%	69%
	Pulpwood Pond	47%	36%	41%
Open Canopy Reference	17 Frog Pond	100%	100%	100%
	Brandon's Pond	100%	100%	100%
	Swain Pond	100%	100%	100%
	Tiger Pond	17%	15%	16%
Enhancement	Block T Pond	100%	49%	75%
	Braswell Pond	100%	100%	100%
	LL Dismal	45%	26%	35%
	Slate Circle	33%	22%	27%
Re-establishment	Dover Bay	100%	100%	100%
	Juniper Bay	45%	33%	39%
	Parker Farms	100%	100%	100%
	Stone Farm	40%	60%	50%

SAMPLING PROTOCOL ANALYSES

Macroinvertebrates

An effort was made during this study to determine an optimal wetland macroinvertebrate sampling protocol. To that end, a variety of sampling methods were used in the first year of the study, to compare results in terms of abundance and diversity of species detected. When possible, a second bucket sample or second shallow sweep was obtained. For all of these samples combined, a total of 30,033 individual macroinvertebrates were identified, representing 304 distinct taxa.

Examination of species data by sample type showed that adding a bucket sample to the initial shallow sweep added an average of 5.7 taxa to the shallow sweep lists (range 0-13 taxa). A second shallow sweep often doubled the total taxa count (added an average of 11 more taxa). Shannon's diversity (H') index showed significantly different biodiversity between a first and second shallow sweep (for all sites where a second shallow sweep was taken except Cypress Pond) (t test $p<0.10$) (Hammer et al. 2001). Biodiversity in the first sample exceeded the second sample in seven of eight cases. A deep sweep, when obtainable, added an average of 5.8 (range 4-9) new taxa; a 2nd deep sweep added between 0 and 8 more taxa.

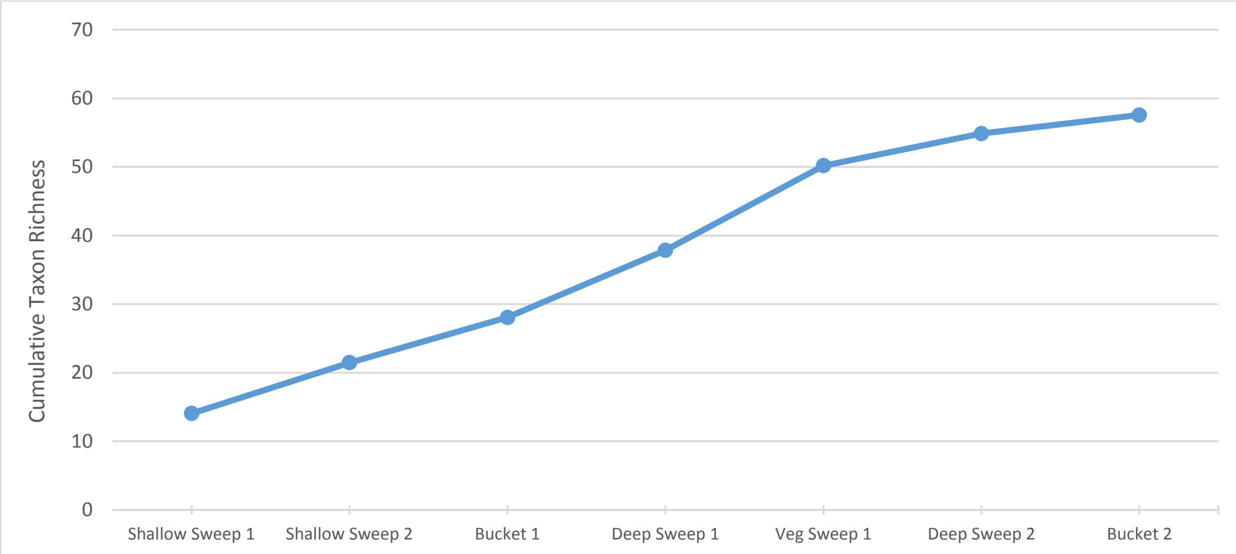
A second bucket sample was taken at seven of the 16 sites in 2013. Although additional species were always detected in a second bucket, a second bucket sample usually added only a few more taxa (1-5). No clear pattern emerged by adding a second bucket sample. On four of the seven sites with a second bucket sample, biodiversity was significantly higher or lower in the second bucket (three higher, two lower) (t test $p<0.10$). Three of the seven sites with a second bucket sample showed no difference in biodiversity between the two bucket samples (t test $p>0.10$) (Hammer et al. 2001). Significant differences in macroinvertebrate abundance between buckets existed for only 2 of the 7 sites (Block O and Juniper Bay). These results underscore the idea that macroinvertebrates in wetlands can have patchy spatial distributions; however, it appears that a second bucket sample was not necessary in most cases.

A species accumulation curve was produced for each site based on the samples which were taken, to show the increase in new species detected with each additional method (Figure 67). Sampling methods were generally ordered (left to right) to produce the greatest increase in new taxa detected from the previous sampling method. This can, in effect, indicate an optimal sampling protocol.

Based on the biodiversity analyses and the species accumulation curve, the recommended protocol would be at least two shallow sweeps and one deep sweep (if possible) along with one bucket sample. Bucket samples also have the advantage of allowing a quantitative measure of density, if desired.

Figure 67. Species accumulation curve by sampling method for macroinvertebrates in study wetlands.

Veg Sweep = sweep with a D net through emergent aquatic vegetation where present.



Amphibians

Amphibians were sampled on all sites using a combination of methods – dipnet/visual egg mass surveys and frogloggers. The resulting data were analyzed to understand how the frogloggers added to the information gathered using dipnets, which cost less in dollars and time. Mann-Whitney U tests showed that using only the frogloggers resulted in significantly higher amphibian species richness and AQAI than through only dipnet surveys across all sites ($p<0.003$) (Figures 68 and 69). There was a significant difference between dipnet only and the combined (froglogger+dipnet) survey results ($p<0.0004$). Dipnetting *in addition to* froglogging did not significantly change the number of species detected or the AQAI (no diff. between froglogger results and froglogger+dipnet results – $p>0.50$).

Amphibian species detection clearly benefited from the use of frogloggers. However, this method comes at a cost, not just for the recorders themselves, but also for the time needed for an expert to listen and record call identity. This project generated 3,193 hours of recordings that were downloaded and listened to by a trained scientist. In 2014, the frogloggers were generally left up and recording every night from January through July on nearly all sites, which generated over 1,500 hours of recording time to review. Perhaps the loggers could have been set to record for a few days each week over the course of the breeding season with similar results in species detection.

If dipnetting is not feasible, frogloggers can yield valuable information; however, it is important to remember that non-calling species (ie. newts and salamanders) cannot be detected by frogloggers but can by dipnetting.

Figure 68. Effect of sampling technique on amphibian species richness detected.

REFCL = closed canopy reference, RE-ESTAB = re-establishment, REFOP = open canopy reference, ENHANCE = enhancement, D = dipnet surveys, L = froglogger, L+D = combined techniques.

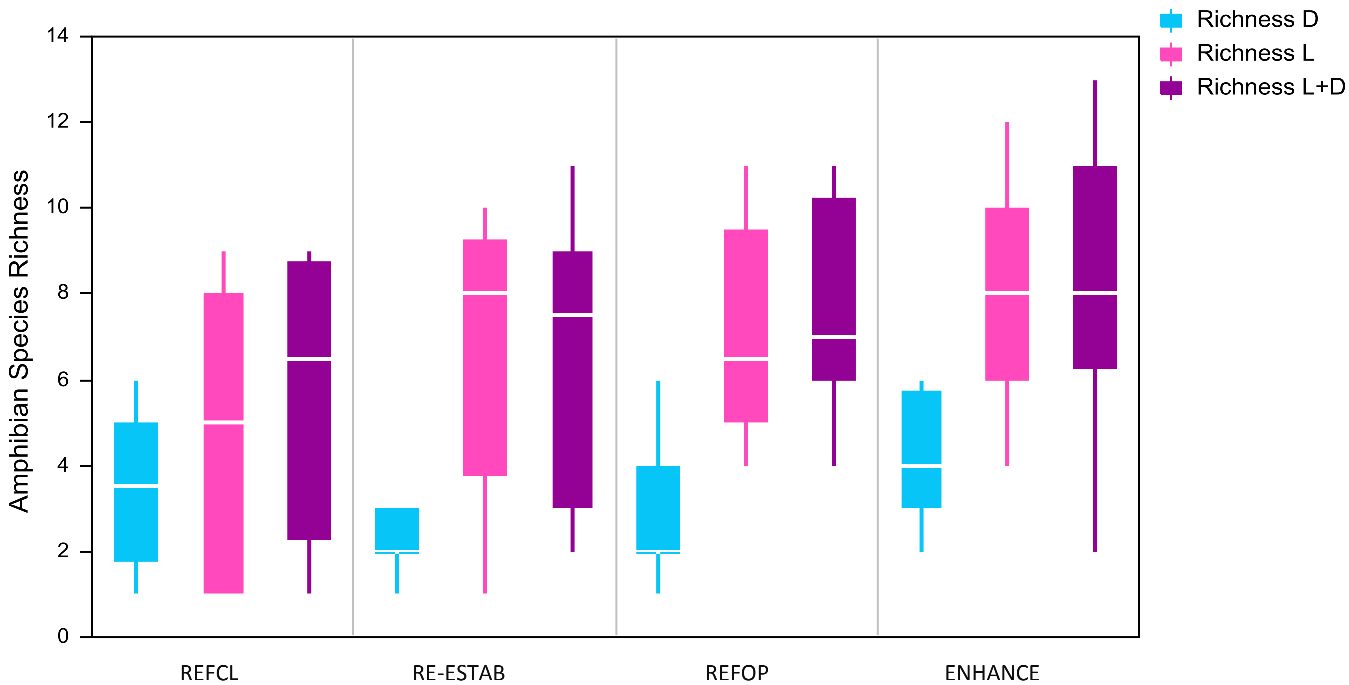
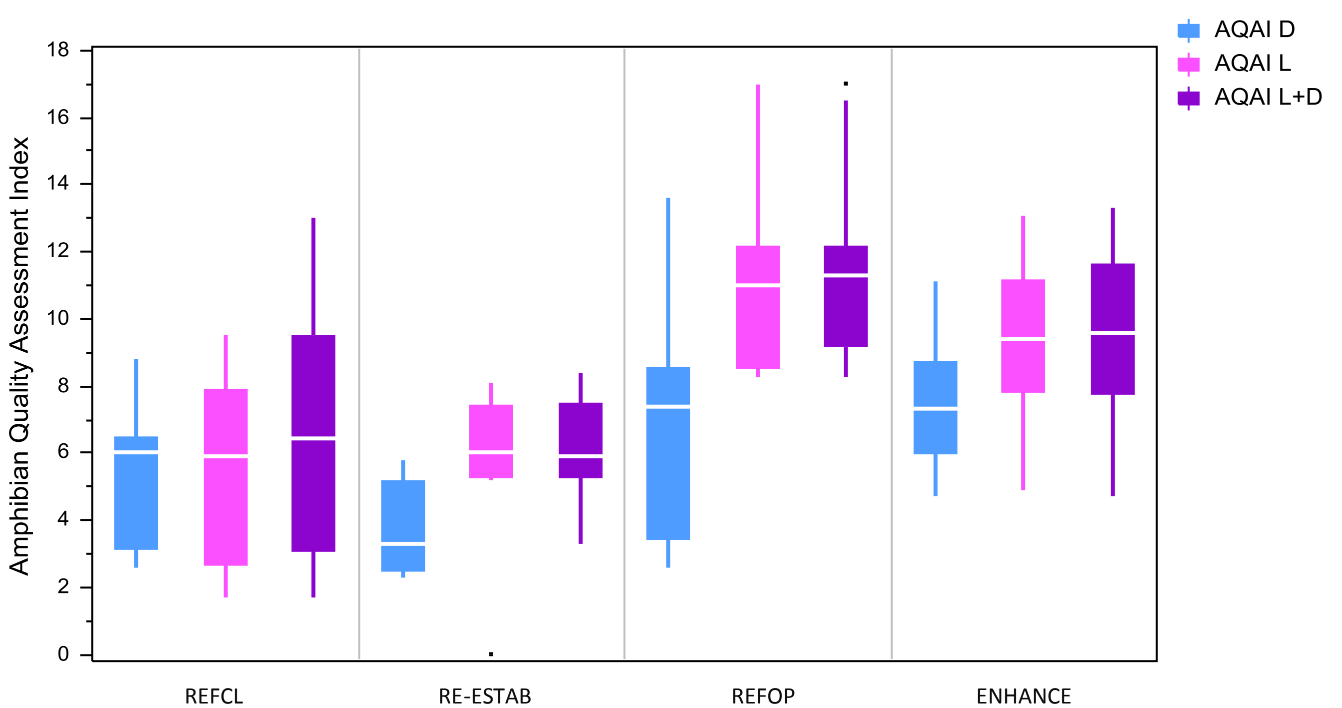


Figure 69. Effect of sampling technique on the Amphibian Quality Assessment Index (AQAI).

D = dipnet surveys, L = froglogger, L+D = combined techniques.



COMPARISONS OF MACROINVERTEBRATE COMMUNITIES ACROSS SITE TYPES

Data from one shallow sweep and one bucket sample from each wetland site in each year were analyzed for comparisons of macroinvertebrate communities across wetland site type (deep sweeps were not able to be obtained from each wetland). From these two sample types, a total of 15,179 individual macroinvertebrates were identified; 4,028 in closed canopy reference sites, 3,308 occurred in re-establishment sites, 2,172 in open canopy reference sites, and 5,671 in enhancement sites. Within the samples, 75 distinct taxa were identified in closed canopy reference wetlands, 133 taxa in re-establishment sites, 97 taxa in open canopy reference wetlands, and 101 taxa in enhancement sites.

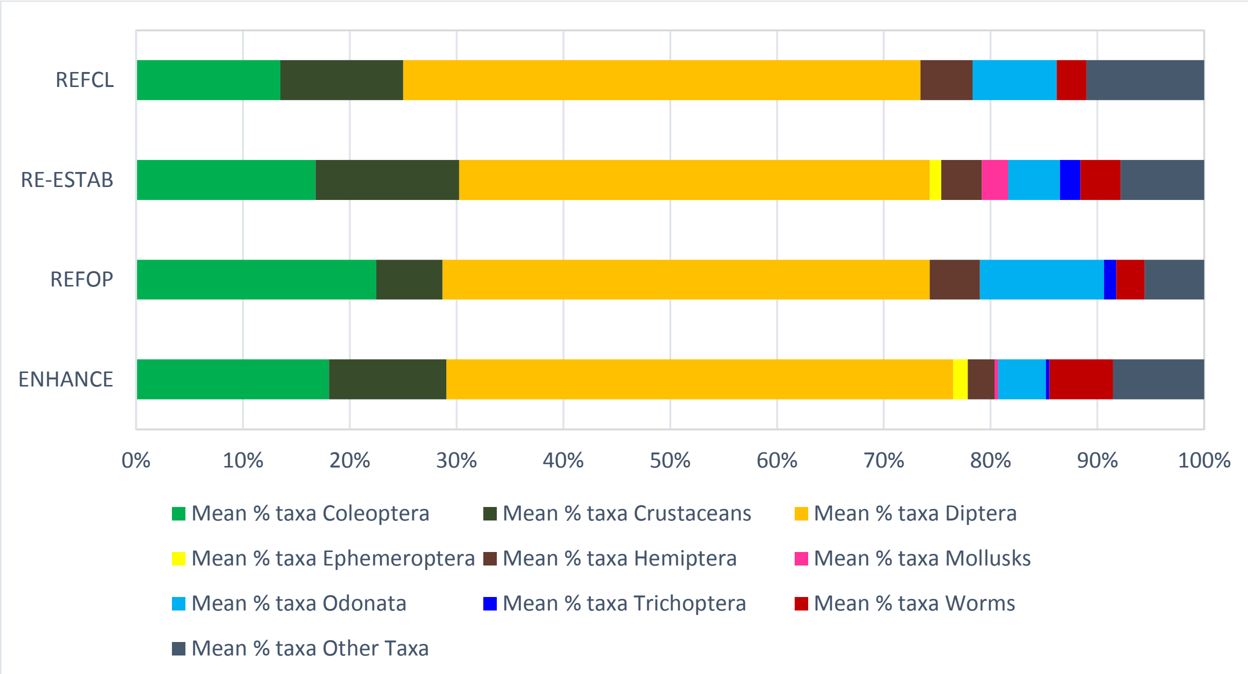
In terms of abundance, the seven most abundant taxonomic groups across all wetlands sampled were Diptera (mostly Chironomidae, Culicidae), Ostracoda, Subclass Copepoda, Isopoda (Asellidae), Amphipoda (Crangonyctidae), Odonata (mostly Coenagrionidae, Libullulidae), and Coeloptera (mostly Dytiscidae, Hydrophilidae, Hydrochidae). West Virginia reference wetlands supported more Isopoda and Odonata than re-establishment wetlands (Balcombe et al. 2005). Their findings relate proportion open water to vegetation/emergent area and found increased macroinvertebrate taxa richness and abundance in vegetated areas. We found no relationship between percent of pool with open water and taxon richness or abundance, but this may be confounded by differences in wetland type.

Microcrustaceans (Copepods and Ostracods) were either the first or second most abundant taxa in each wetland type. Larger crustaceans (Amphipods and Isopods) were the third most abundant taxa in each wetland type except the open canopy reference wetlands, where they were virtually absent. All other site types had lower dissolved oxygen, standing water cover, and aquatic plant cover than the open canopy reference wetlands, which could have been a factor (Figures 80 and 81).

On all site types, Dipterans represented the largest portion of the taxa, followed by Coleopterans and Crustaceans (except on open canopy reference wetlands, where Odonate taxa were more numerous than crustacean taxa) (Figure 70). Phylum Mollusca was nearly always found only on re-establishment sites, with a few found on enhancement sites and none in reference wetlands. Water was more acidic in reference wetlands than in enhancement and re-establishment sites (Figure 88). Mean pH of all 12 sites that had no mollusks was 3.93 and mean pH of the four sites with mollusks was 4.53 (Spearman's $p=0.004$). In a depressional marsh in Florida, Denson (undated) also found very few mollusk taxa at a mean pH of 5.77. In acidic waters, mollusks are unable to maintain shell calcium.

Mayflies (Order Emphemoptera) were only observed on re-establishment and enhancement site, but not on any reference sites. Mayflies are also sensitive to pH, but we did not find a significant correlation between Mayfly presence and mean pH in this study (Spearman's $p=0.14$). Crayfish showed a similar pattern: they were found only on re-establishment and enhancement site and on no reference sites, but this did not appear to be related to pH, at least within the pH ranges on our study sites (Spearman's $p=0.42$).

Figure 70. Proportions of total taxa from each macroinvertebrate order by site type.



Several taxa were only found on re-establishment sites, and not in the other wetland types. These included worms, the beetle family Dytisidae, and the beetle family Haliplidae, which is associated with aquatic vegetation. The presence of these groups may have been related to the extended (or permanent) inundation of the re-establishment sites, as compared to the other site types, which often showed strong seasonal patterns of inundation and drying. A significant dissimilarity in taxonomic composition was observed between re-establishment sites and closed canopy reference sites (ANOSIM p=0.031).

The Tanytarsini midges were never found on the closed reference wetlands, but were found on the other three site types. This may be due to a lack of wind-driven water circulation in closed canopy sites, because these midges are filter feeders and rely on the movement of water to feed. Denson (undated) also found low levels of filterers like the hydropsychid caddisflies and scrapers such as the mollusks and certain beetles in an isolated depressional wetland in Florida.

SIMPER analysis in PAST statistical software (Hammer et al. 2001) was used to calculate the similarity percentage based on the Bray-Curtis similarity index between re-establishment sites and closed canopy reference sites, in terms of macroinvertebrate taxa (family level) and abundance. (Abundance data were square root transformed, to reduce the effect of abundance extremes.) This method was also used to assess which taxa were primarily responsible for the difference between the site types (Clarke 1993). The re-establishment sites and closed canopy reference sites were 78% dissimilar in their taxonomic composition, the biggest contributors to this being differences in presence and abundance in midges (Chironomidae), copepods, isopods (Asellidae), amphipods (Crangonyctidae), ostracods, and aquatic worms (Naididae). Midges and copepods were much more abundant at closed canopy reference sites, while asellid isopods, amphipods, ostracods, and aquatic worms were much more abundant at re-establishment sites. Copepods were entirely absent from re-establishment sites (mean abundance = 462 at closed canopy reference sites).

The enhancement sites and open canopy reference sites were 78% dissimilar in their taxonomic family composition, mostly driven by presence and abundance differences in midges (Chironomidae), ostracods, asellid isopods, and damselflies (Coenagrionidae). All were more abundant at the enhancement sites, except the damselflies, which were 25 times more abundant at the open reference sites.

Incidentally, the two types of reference sites were 80% dissimilar, mostly due to greater abundances of copepods, amphipods, and some species of midges at the closed canopy reference sites, and greater abundances of ostracods, damselflies, and Psectrocladius midges at the open canopy reference sites. Amphipods were absent and copepods were found in extremely small numbers on open canopy reference sites.

When analyzed at the genus level, the macroinvertebrate assemblages on re-establishment sites and closed canopy reference sites showed a higher level of dissimilarity than when analyzed at the family level (85%). This difference was driven by the fact that Copepods and the midge Camptocladius were both abundant on the closed canopy reference sites and absent on the re-establishment sites altogether. The midge Polypedilum was also found in much greater abundance on the closed canopy reference sites than on the re-establishment sites. The re-establishment sites had substantially more amphipods (*Crangonyx*), asellid isopods, and ostracods than the closed canopy reference sites.

The macroinvertebrate assemblages (genus level) on the enhancement sites and open reference sites were 83% dissimilar, this being driven by much greater numbers of ostracods, asellid isopods, midges (*Psectrocladius*, *Polypedilum*, *Natarsia*), and megadrile worms at the enhancement sites. The midge Ablabesmyia and hydrophilid beetle (*Hydrochus*) were present in greater numbers at the reference sites.

Re-establishment sites had higher numbers of macroinvertebrate taxa, abundance, and density than other sites types, probably due to connections to permanent water (Figures 71 and 72). Open canopy reference sites had less variation in abundance across taxa (species evenness) than other site types. In general, all wetlands had much lower proportions of sensitive taxa than tolerant taxa (Figure 73). Macroinvertebrate Biotic Index (MBI) values also tended toward the tolerant end of the spectrum for the study wetlands. This is consistent with the idea that stressful conditions naturally occur in seasonal wetlands and macroinvertebrates present reflect those conditions. This is not necessarily related to pollution levels. To be useful in wetlands, the MBI developed for stream bioassessment may need to be modified for wetlands where stressful conditions naturally occur, especially in seasonal wetlands.

Figure 71. Median and range of macroinvertebrate abundance and density by site type.

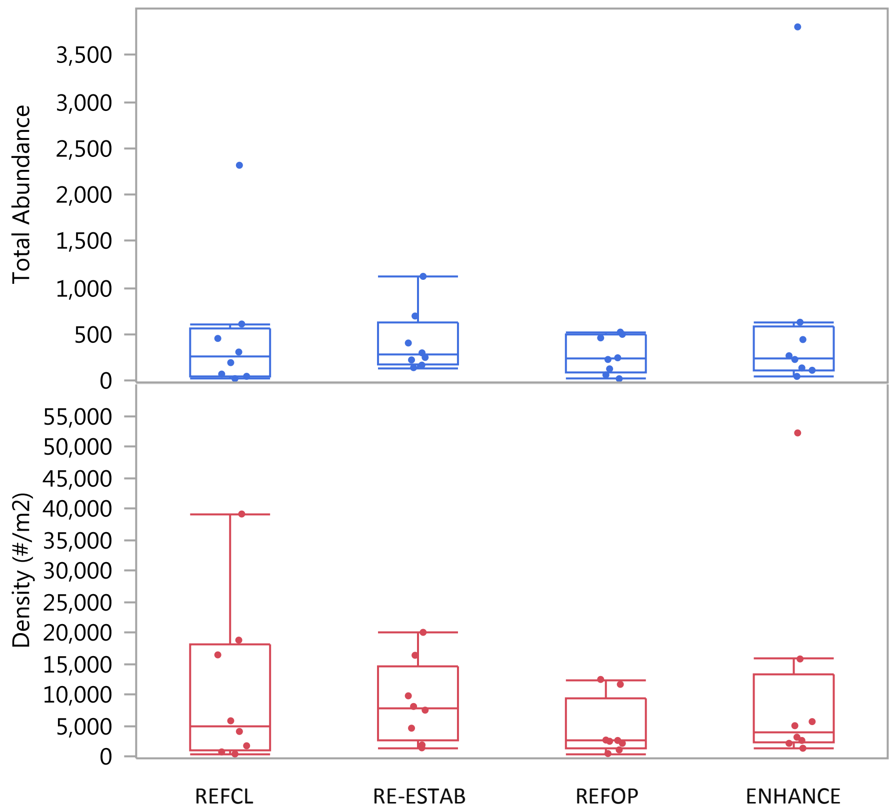


Figure 72. Macroinvertebrate taxon richness, diversity, and evenness by site type.

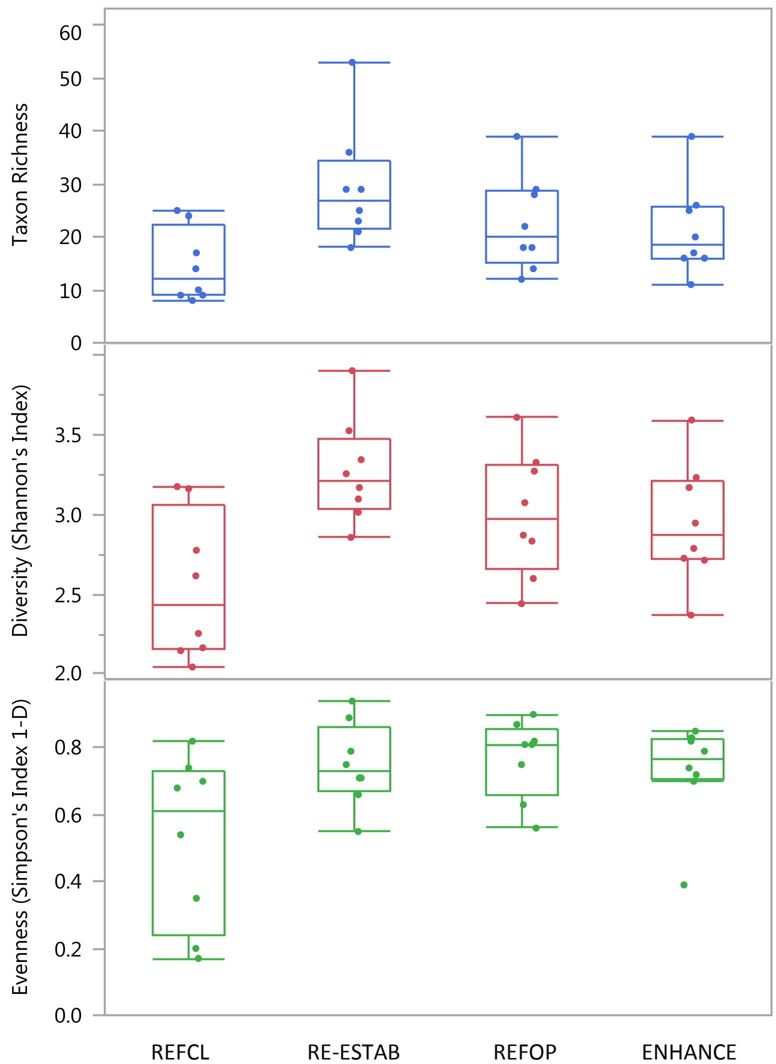
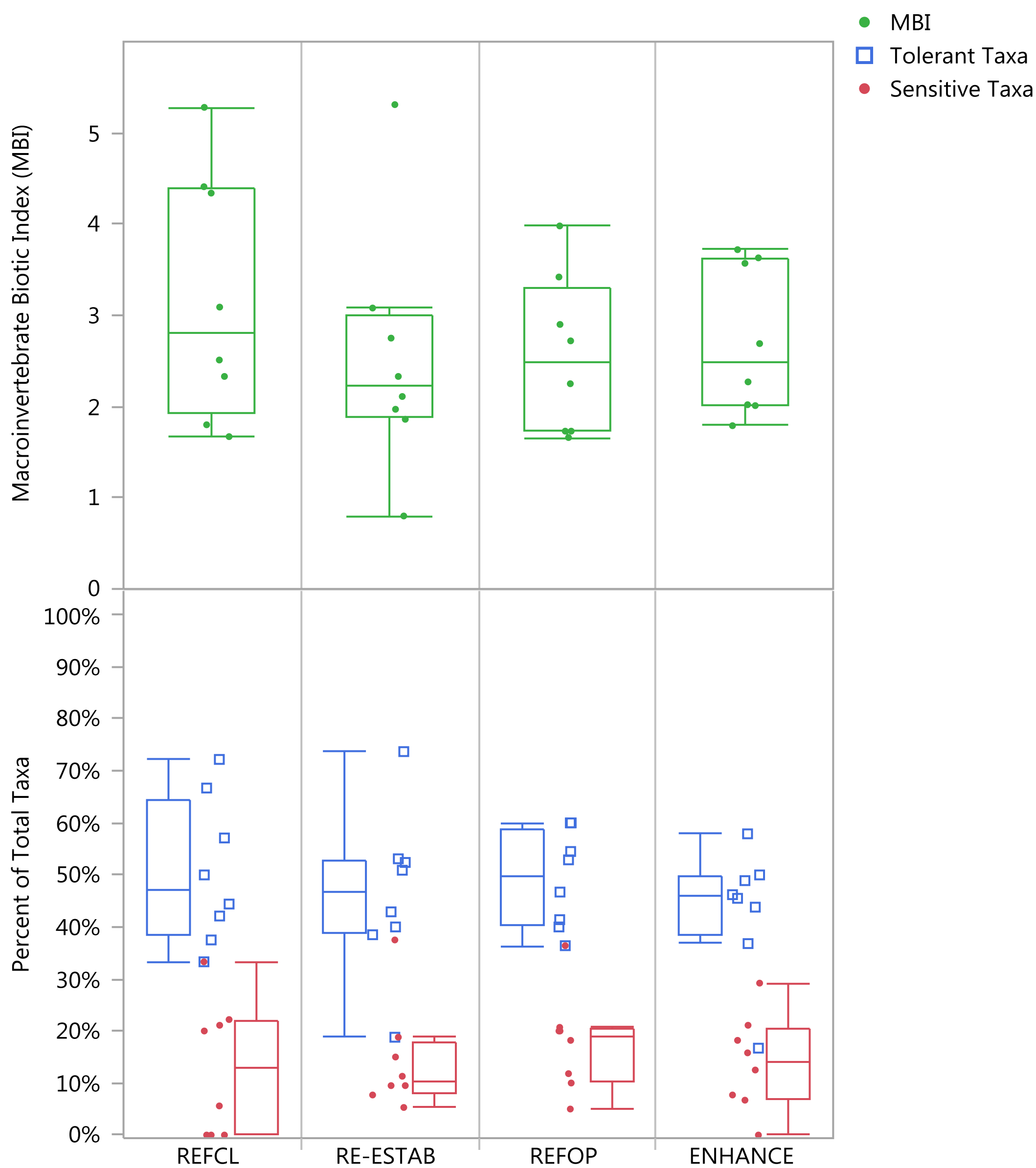


Figure 73. Macroinvertebrate Biotic Index value, percent sensitive taxa, and percent tolerant taxa by site type.



Comparison of Highest and Lowest Ranked Sites

Sites were ranked from best to worst according to macroinvertebrate taxon richness and total macroinvertebrate abundance (1 being best; 16 being worst) (Table 33). It should be noted that all sites in this study were located in rural areas and surrounded by fairly low levels of human impact, so they do not represent a complete spectrum of best and worst possible in North Carolina. This ranking was done to further elucidate variables that may relate to wetland habitat quality in general for macroinvertebrates.

Sites ranked best and worst differed significantly in macroinvertebrate density, taxon richness, and abundance (Figure 74). Of the 16 sites in this study, sites with the best rankings (rank of 1-5) were re-establishment sites and open canopy reference sites. The middle rankings (ranks of 6-11) and worst rankings (12-16) were found in all site types.

A closer look at site rankings and habitat factors can show how sites with a rich macroinvertebrate presence differ from those with fewer macroinvertebrates. Of the myriad habitat variables that were measured for this study, we graphed each individually in a preliminary analysis to find those variables that differed markedly between the best and worst ranked sites. The results showed that the best sites had longer inundation times, more sphagnum moss cover, more aquatic plant cover, and more coniferous tree cover in the wetlands (Figure 75). The best wetlands also had more vegetation size classes present, lower species richness of dominant species, and buffers with greater broadleaf tree cover and vegetation strata (Figure 76).

Table 33. Site rankings based on macroinvertebrate abundance and taxon richness.

Sites with best rankings have a lower mean rank number and are darker green, middle rankings are blue, and worst rankings are orange. Sites are sorted by site type: RE-ESTAB = re-establishment sites, REFCL = closed canopy reference sites, REFOP = open canopy reference sites, ENHANCE = enhancement sites

Site Name	Site Type	Average of Total Macro Abundance	Average of Macro Taxon Richness	Abundance Rank	Richness rank	Mean Rank
Block T Pond	ENHANCE	2123	32.5	1	3	2
Swain Pond	REFOP	492	33.5	4	2	3
Dover Bay	RE-ESTAB	432	37	7	1	4
Parker Farms	RE-ESTAB	711	24	3	6	4.5
Cypress Pond	REFCL	325	24.5	9	5	7
Juniper Bay	RE-ESTAB	237.5	32.5	11	4	7.5
Brandon's Pond	REFOP	373	23.5	8	7	7.5
Pulpwood Pond	REFCL	1169.5	12.5	2	14	8
Braswell Ponds	ENHANCE	449	16.5	6	11	8.5
Stone Farm	RE-ESTAB	273.5	23.5	10	8	9
Block O Pond	REFCL	459.5	12	5	15	10
Little Little Dismal Pond	ENHANCE	183.5	22.5	12	9	10.5
17 Frog Pond	REFOP	95.5	17	14	10	12
Tiger Pond	REFOP	125.5	16	13	12	12.5
Slate Circle	ENHANCE	80	13.5	15	13	14
Gum Pond	REFCL	60	9	16	16	16

Figure 74. Differences in macroinvertebrate density, taxon richness, and abundance in sites with worst and best rankings.

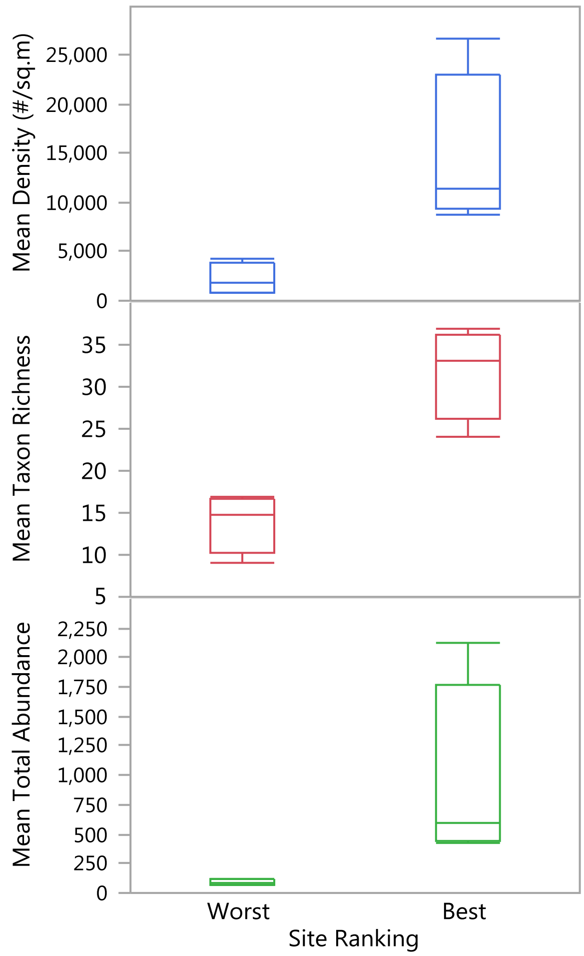


Figure 75. Selected habitat variables with differences between worst and best ranked macroinvertebrate sites.

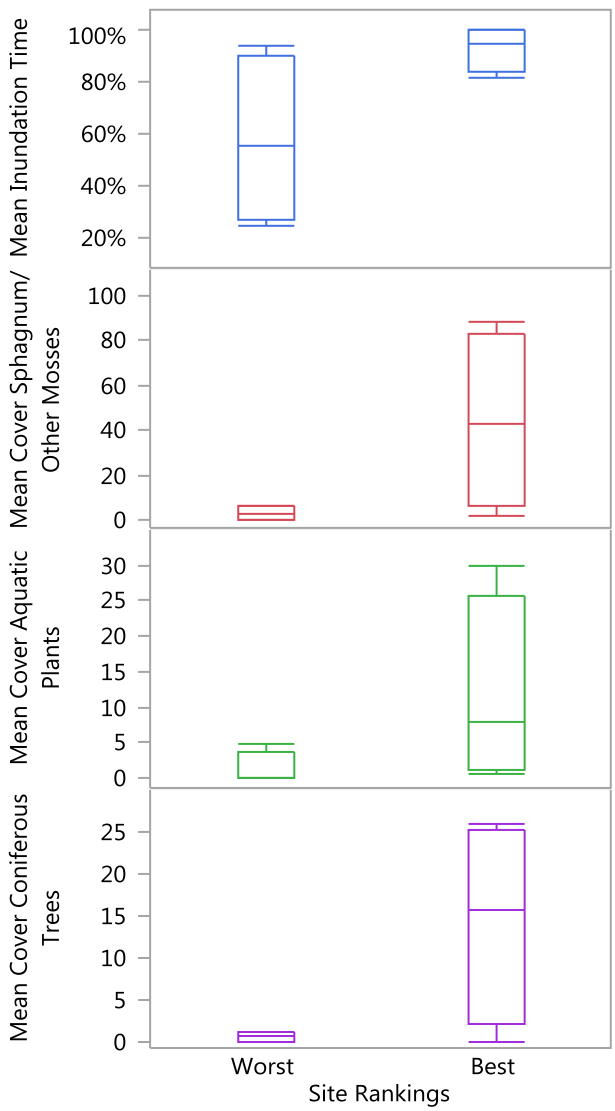
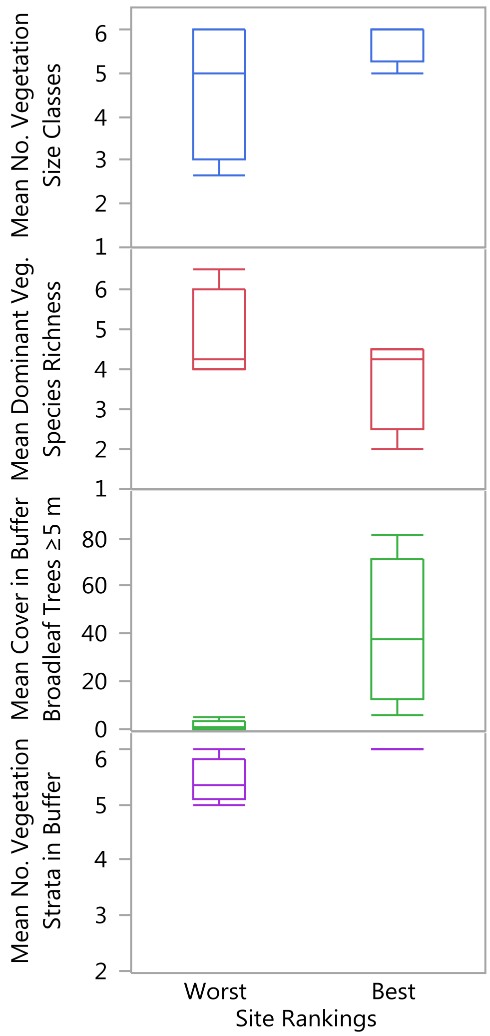


Figure 76. Selected wetland vegetation and buffer variables with differences between worst and best ranked macroinvertebrate sites.



Multivariate Analysis of Factors Influencing Macroinvertebrate Communities

Analysis of factors influencing macroinvertebrate communities was conducted using Canonical Correspondence Analysis (CCA). The CCA is a multivariate constrained ordination technique that extracts major gradients among combinations of explanatory variables in a dataset. It places study sites in a dimensional space in relation to each other based on a similarity index. Environmental variables are also included in the analysis to reveal which measured environmental variables significantly relate to the species/site associations. The lengths of the lines in the resulting plot indicate the strength of the influence.

Macroinvertebrate community metrics (Table 34) were correlated using Spearman's non-parametric correlation with 73 habitat, landscape, and amphibian variables. This resulted in a list of 47 environmental variables that significantly related to the macroinvertebrate metrics ($p<0.10$). This list of 47 environmental variables was tested for autocorrelation, to discern which environmental variables highly correlated with each other (Spearman's $r>0.50$ and $p< 0.10$). In the end, 13 variables were found to correlate with one or several macroinvertebrate metrics and not highly correlate with each other (Table 35). These 13 variables were chosen to initial input into the CCA. Figure 77 shows the direction and strength of the correlations of the 13 macroinvertebrate community metrics and the environmental variables. Correlations varied in direction and strength.

Table 34. Macroinvertebrate metrics which were tested for correlations with all habitat and landscape variables.

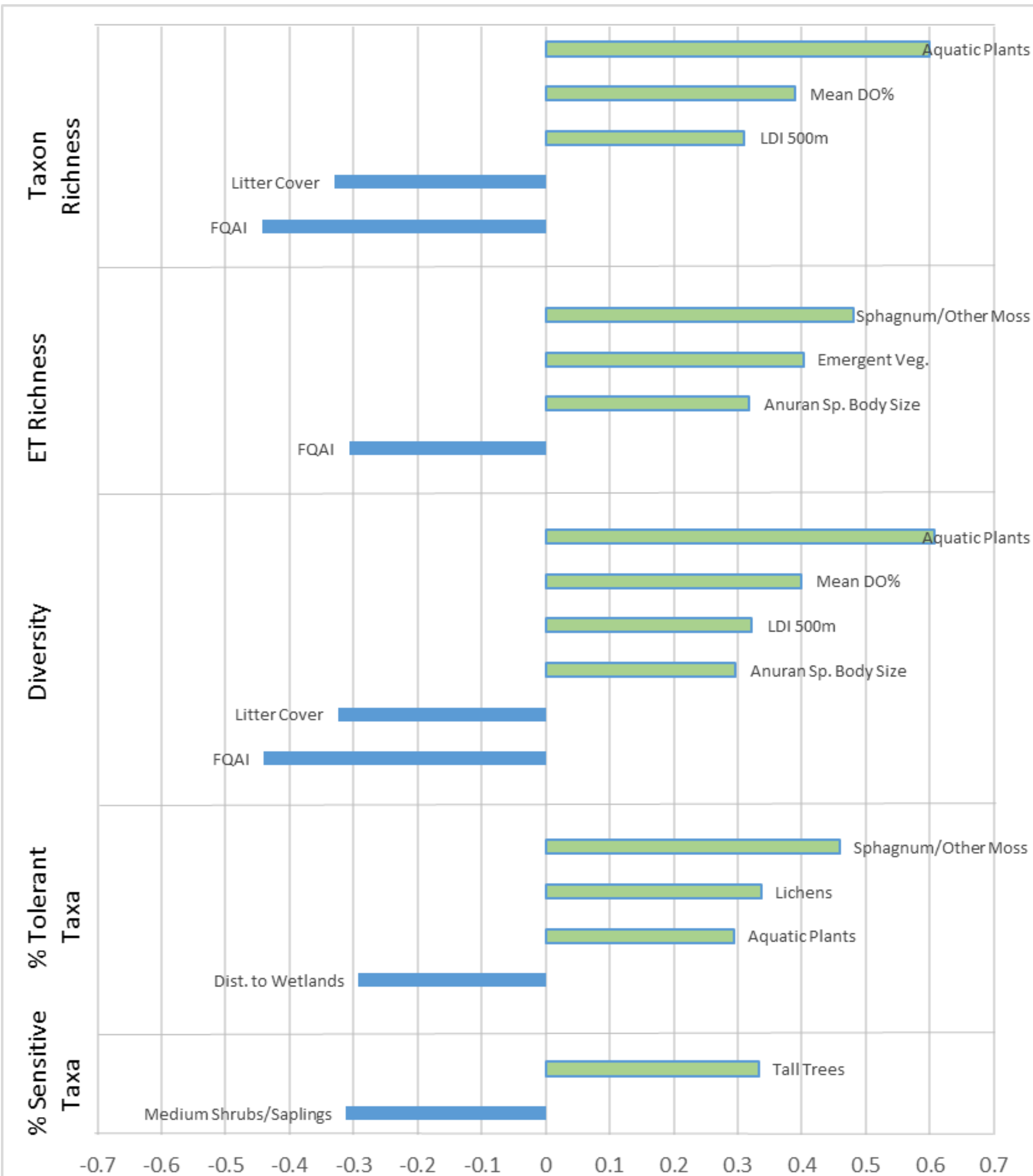
Macroinvertebrate Metrics Tested for Correlations	
Total Taxon Richness	Macroinvertebrate Biotic Index (inverted)
Total Abundance	% Tolerant Taxa (Tolerance Value >7.5)
Density	% Sensitive Taxa (Tolerance Value <4.4)
Diversity (Shannon's Index H')	Ephemeroptera + Trichoptera Richness
Evenness (Simpson's Index 1-D)	

Table 35. Environmental variables found to be significantly correlated with one or more macroinvertebrate metrics across all site types *and* not highly correlated with each other.

Environmental Variables Significantly Correlated with One or More Macroinvertebrate Metrics	
LDI 500m	Lichen Cover
Mean Distance to Nearest Two Natural Wetlands	Dead Standing Small Shrub/Sapling Cover (<5cm dbh)
Tall Trees (>30m) Cover	Total Litter Cover
Medium Shrubs/Saplings Cover (0.5m – 5m)	FQAI
Emergent Vegetation Cover	Mean Dissolved Oxygen (DO) (% Saturation)
Aquatic Plant Cover	Anuran Species Body Size
Sphagnum/Other Moss Cover	

Figure 77. Spearman's correlation coefficients showing direction and strength of relationship for variables that were significantly correlated with selected macroinvertebrate metrics (p<0.10) *and* not correlated with each other.

ET Richness = Number of Ephemeroptera + Trichoptera taxa, Mean DO% = Dissolved Oxygen (% Saturation), LDI 500m = Landscape Development Intensity Index (500m around wetland), FQAI = Floristic Quality Assessment Index, Amphibian Sp. Body Size = mean adult body size for amphibians detected in wetland, Tall Trees = cover of trees >30m tall, Medium Shrubs/Saplings = shrubs and saplings ≥0.5 – 5m tall.



The CCA model resulted in a significant p value, which showed that the observed relationships between the environmental variables is not due to chance. Variables with the least influence (as indicated by lengths of lines in the CCA plot) were dropped to produce a model with axes that explained more variation in macroinvertebrate taxa occurrence. The resulting significant CCA included five important environmental variables: aquatic plant cover, moss cover (mainly Sphagnum), emergent vegetation cover, tall tree cover (>30m), and dominant species FQAI. (CCA overall $p = 0.002$; 62.9% of variance explained; Axis 1 – 35.5%, Axis 2 – 27.4%). Enhancement sites and open reference were similar with regards to these five factors and the re-establishment and closed reference sites differed from each other (Figure 78).

This CCA was performed using a cumulative occurrence of macroinvertebrate taxa (at the genus level) and means of environmental variables for years 1 and years 2 of the study (because macroinvertebrate data were only able to be obtained in years 1 and 2). Genus level taxonomic analysis was done after the recommendation of King and Richardson (2002); CCA was also performed at the family and order level, but it separated site types more clearly at the genus level. These results are similar to those found by Lillie (2003) in Wisconsin isolated depressional wetlands, where water duration was the second most important factor influencing macroinvertebrate community composition (alkalinity being the first). Aquatic plant cover can be used as a proxy for water duration in interpreting our study results in comparison, since they were highly correlated. Unlike the Wisconsin study, we did not find a relationship between macroinvertebrate richness and pH, perhaps because all sites were quite acidic (mean pH ranging from 3.43 to 5.51) and did not represent the full range found in wetlands. However, it is still noteworthy that even at very low pHs, macroinvertebrates were found, sometimes in large numbers.

The results of the CCA and site rankings suggest that the best sites for macroinvertebrates have aquatic plants, a moderate FQAI, moderate emergent vegetation, and few tall trees. A biplot of the CCA results showing taxa and environmental variables reveals which taxa tended to be present under what environmental conditions (Figure 79).

Figure 78. Biplot of Canonical Correspondence Analysis results showing site groupings and environmental variables on macroinvertebrate species occurrence (at the genus level).

Site rankings (1=best; 16=worst) in terms of macroinvertebrate taxon richness and abundance are given before site names. (CCA overall p = 0.002; 62.9% of variance explained; Axis 1 – 35.5%, Axis 2 – 27.4%). RE-ESTAB = re-establishment sites, REFCL = closed canopy reference sites, REFOP = open canopy reference sites, ENHANCE = enhancement sites

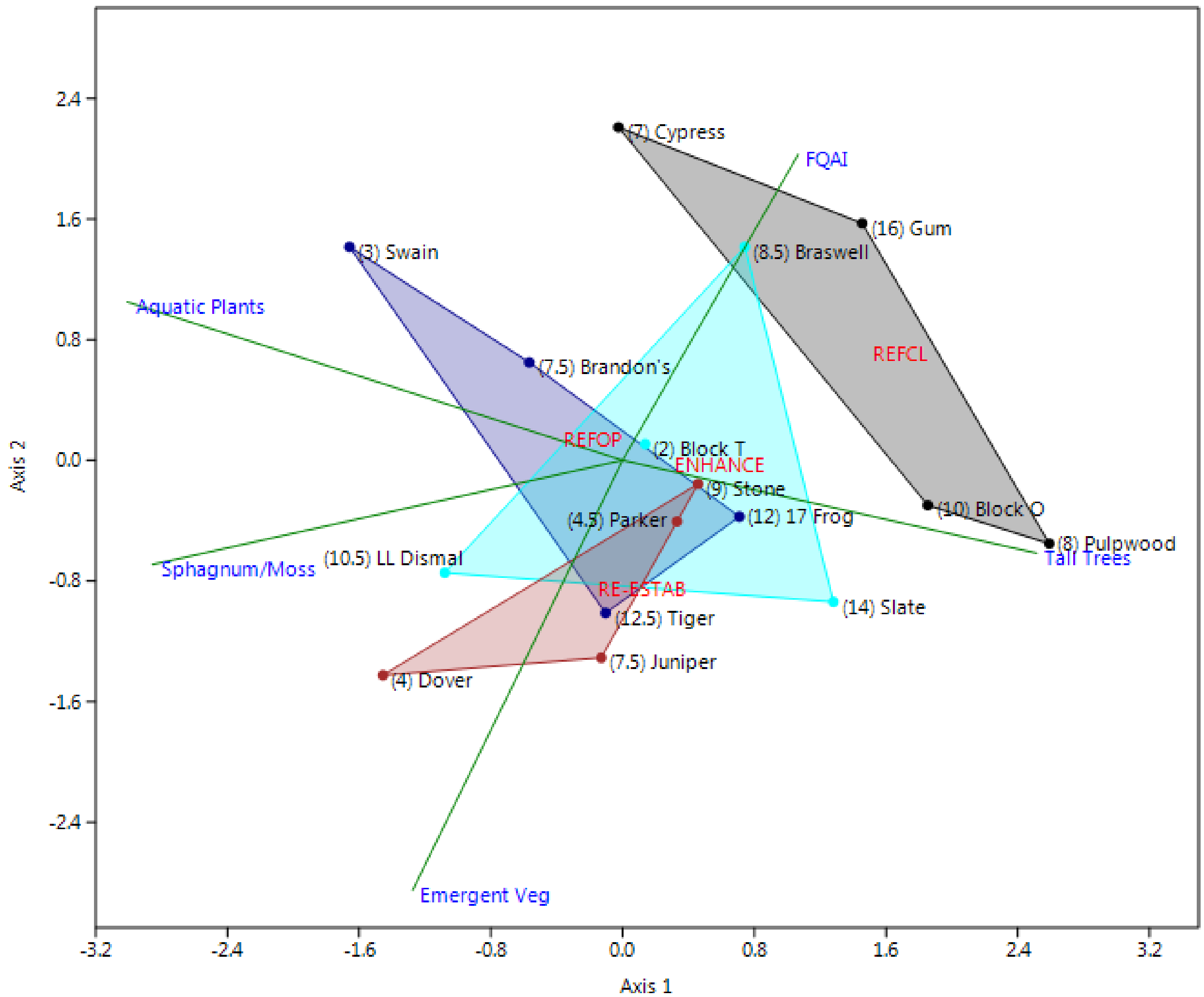
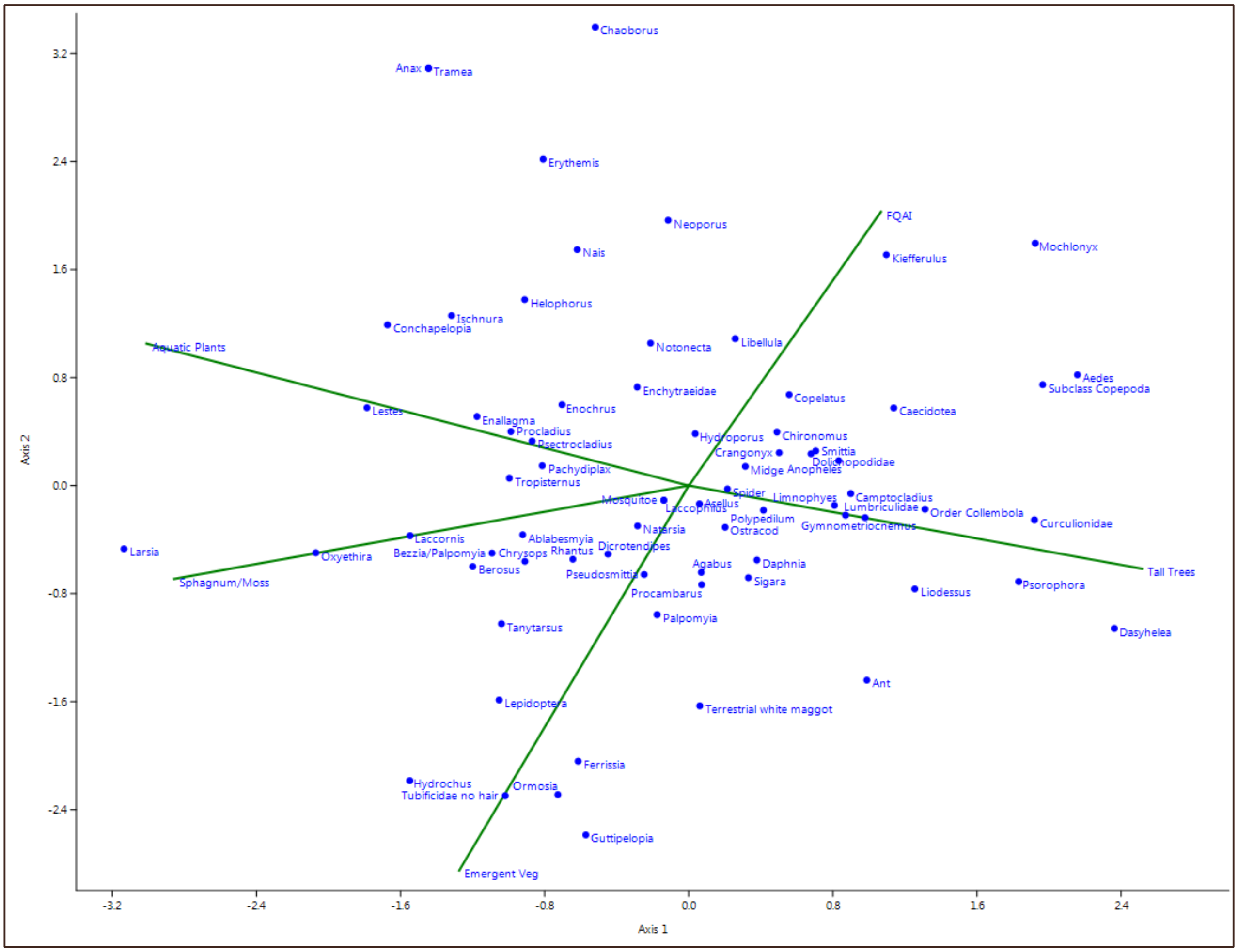


Figure 79. Biplot of Canonical Correspondence Analysis results showing taxa and environmental variables on macroinvertebrate species occurrence (at the genus level).

Taxa occurring on fewer than 3 sites were not included in the analysis. (CCA overall p = 0.002; 62.9% of variance explained; Axis 1 – 35.5%, Axis 2 – 27.4%)

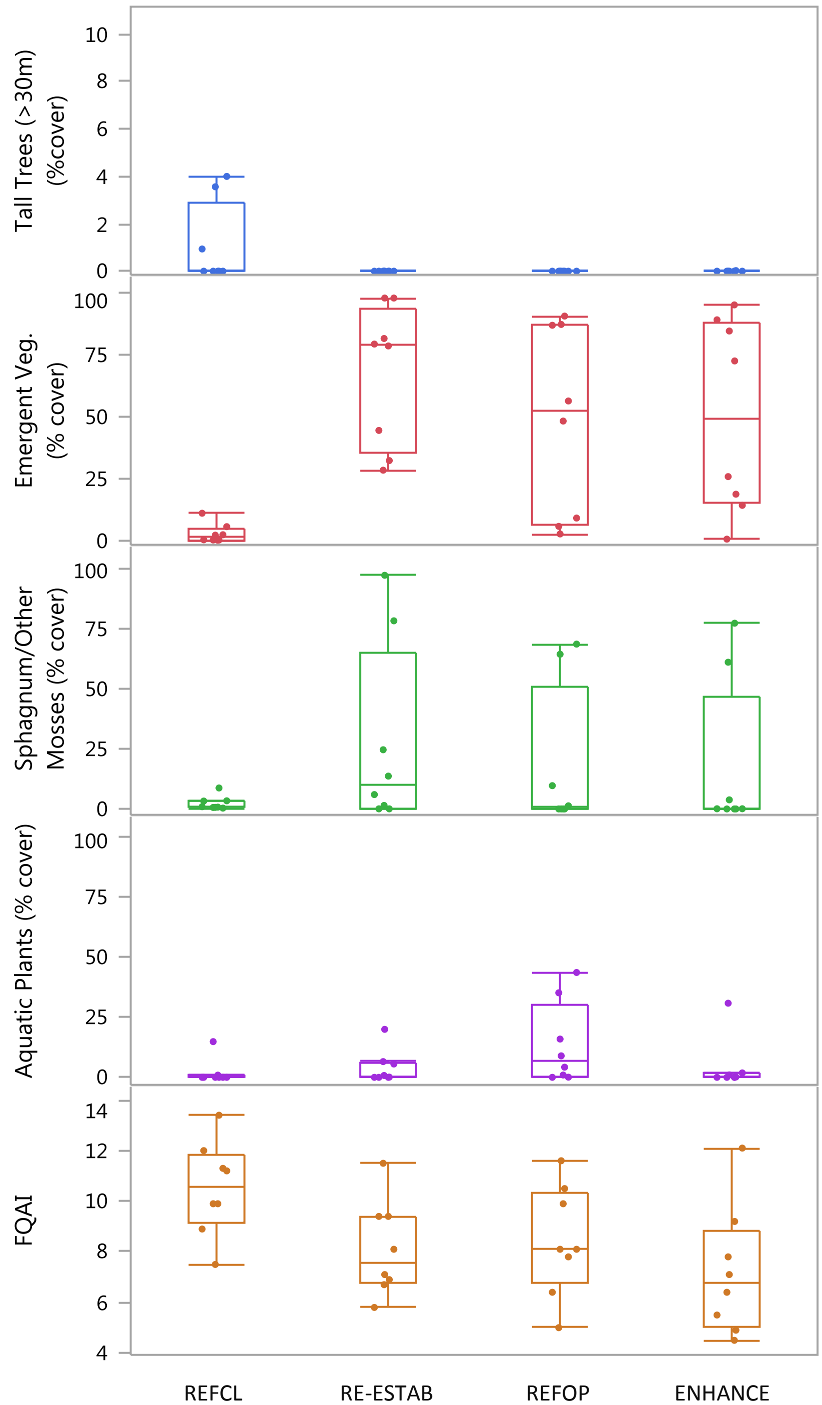


The major environmental variables found to influence macroinvertebrate taxa occurrence on the study sites were: cover of tall trees (>30m height), emergent vegetation, sphagnum (and other) moss, aquatic plants, and FQAI (Figures 78-79). Often one site type stood out as different from the others in these aspects, ie. closed canopy reference wetlands had the tallest trees, re-establishment sites had more emergent vegetation cover and sphagnum moss, open canopy reference sites had the most aquatic plant cover, and closed canopy reference wetlands had the highest dominant species FQAI (Figure 80).

In comparison to closed canopy reference sites, re-establishment sites had significantly lower cover of tall trees (>30m height) (Wilcoxon test, p=0.076), and significantly higher emergent herbaceous plant cover (Wilcoxon test, p=0.001). No difference was detected in sphagnum moss cover or aquatic plant cover between re-establishment sites and closed canopy reference sites (Wilcoxon test, p>0.10). However, overall floristic quality was lower on re-establishment sites than closed canopy reference sites (Wilcoxon test, p=0.031).

In comparison to open canopy reference sites, enhancement sites were no different in terms of tall trees, emergent plant cover, sphagnum moss cover, or overall floristic quality (Wilcoxon test, $p>0.10$). Aquatic plant cover was significantly higher in open canopy reference sites than enhancement sites (Wilcoxon test, $p=0.099$).

Figure 80. Box plots by site type of vegetation related variables found to have important influence on macroinvertebrate community composition (2013 and 2015 combined).



COMPARISON OF MACROINVERTEBRATE FINDINGS TO LITERATURE

Many fish and invertebrates show stress at dissolved oxygen (DO) levels lower than 5 mg/L (Wetzel, 1983). During three years of water quality sampling at our study wetlands, the closed canopy reference and re-establishment sites had mean DO levels at or lower than 5 mg/L (mean 4.6 mg/L for closed canopy reference; mean 4.3 mg/L for re-establishment sites) (Figure 81). The open canopy reference and enhancement sites had higher DO levels (mean 8.6 for open canopy reference; mean 7.1 mg/L for enhancement). Overall mean percent saturation for DO on all closed canopy reference sites ranged from 38% to 52% (year to year), and 24% to 69% in the re-establishment sites from year to year. This contrasts with the open canopy reference sites, which overall ranged from 82% to 99% mean saturation from year to year, and the enhancement sites, which ranged from 64% to 75% mean saturation from year to year. We found a significant positive relationship between dissolved oxygen and macroinvertebrate taxa richness (Spearman's p=0.027) (Figure 82), similar to results found by Nelson et al. (2000) and Tarr et al. (2005).

Extremes in water chemistry and hydroperiod have been shown to cause shifts in macroinvertebrate communities composition (Euliss et al 1999, Schneider 1999, and Golladay et al 1997), but less than extreme variation has been insufficient to show strong relationships (Batzer et al. 2004). This is similar to our results where no difference in community structure was detected between wetlands with hydroperiods varying from 24% to 55% inundation time. More or less permanent wetlands (79%-100% inundation time) had different macroinvertebrate communities than temporary wetlands. Lillie (2003) and Meyer and Whiles (2008) also found hydroperiod length to be an important environmental variable influencing macroinvertebrate community composition.

Our results show significant positive relationships between hydroperiod length and macroinvertebrate taxon richness (but not abundance) when fish were absent (p=0.019) (Figure 83). No relationship was detected on sites where fish were present, but sample size was small. Others have also found, in habitats without fish, invertebrate diversity and abundance increased with hydroperiod (Duffy 1999, Wissinger et al. 1999, Brooks 2000; Tarr et al. 2005). Correlations with increased inundation are consistent with the results of Batzer et al. (2004) and Whiles and Goldowitz (2005). However, the general impact of fish presence on invertebrates is mixed, with some taxa benefiting from fish presence and some not (Wilcox 1992, Pierce & Hinrichs 1997, Batzer et al. 2000, Zimmer et al. 2000).

A study in Minnesota ponds showed that macroinvertebrate taxon richness decreased slightly with increasing pH (Batzer et al. 2004). We found no relationship between taxon richness and pH (with or without fish); however all of our sites had a very restricted pH range lower than 5.5 (Figure 84). In Batzer et al.'s study (2004), taxon richness peaked at pH 6 (though no ponds were lower than 5.5 pH, which was the highest mean pH in our study). In our study, mollusks were primarily found on re-establishment sites, which had the highest median pH levels of all sites. Snails were completely absent from the lowest pH sites, similar to results found by Evans (1996) in acidic Florida marshes.

Figure 81. Mean DO (mg/L) by site type.

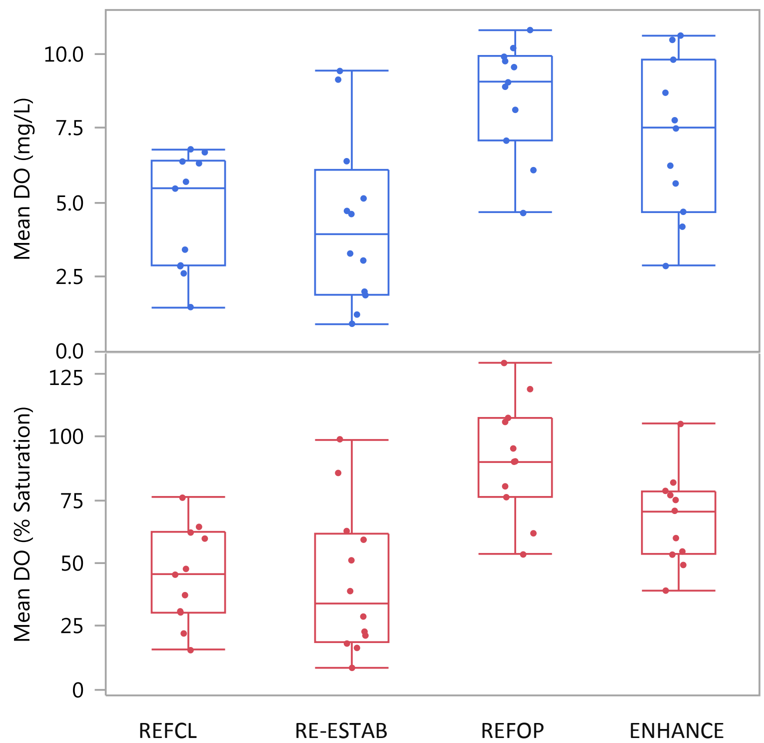


Figure 82. Significant positive correlation between dissolved oxygen and macroinvertebrate taxon richness (p=0.027).

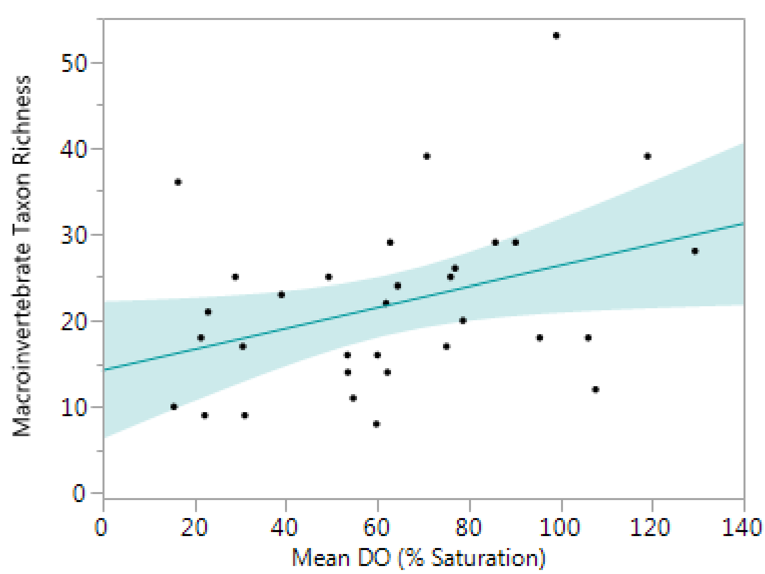
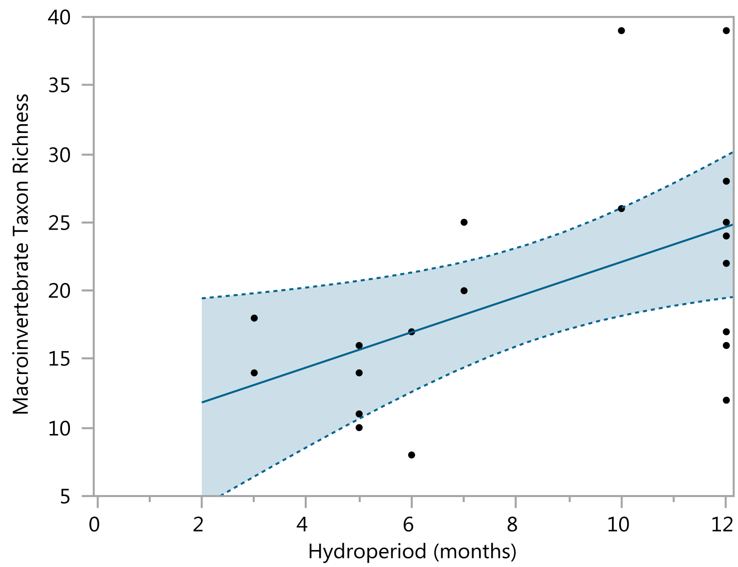


Figure 83. Significant positive relationship between macroinvertebrate taxon richness and hydroperiod length in wetlands without fish (p=0.019).



The presence of vegetation in a wetland has been found to be correlated with higher macroinvertebrate diversity (Morrison and Bohlen 2010; DeSzalay and Resh 2008). We found cover of aquatic plants to be positively correlated with macroinvertebrate diversity and overall taxon richness (Figure 77). Emergent vegetation cover and sphagnum moss cover were positively related to taxon richness of more sensitive taxa (Ephemeroptera and Trichoptera).

Batzer et al. (2004) found no relationship between pond surface area and taxon richness, but a negative relationship of richness to increasing canopy cover (and litter input). Plenzler (2012) concluded that canopy cover over vernal pools impacted macroinvertebrate diversity and productivity. In in his study, vernal pools with the least amount of canopy cover (corresponding with the greatest algal productivity) had the highest macroinvertebrate abundance, family richness, and diversity. Similarly, we found a negative relationship between taxon richness and canopy cover, with or without fish (Figure 85).

Despite these relationships, Batzer et al. (2004) concluded that variation in macroinvertebrate richness and abundance was only weakly explained by environmental variables, and that wetland taxa are adapted to and tolerant of environmental variation. Most successful macroinvertebrates in wetlands may be habitat generalists (Danks and Rosenberg 1987, Euliss et al. 1999, Tangen et al. 2003), and occurrence in wetlands may be more subject to stochasticity than environmental variation.

Figure 84. Lack of relationship between pH and macroinvertebrate taxon richness (p = 0.71).

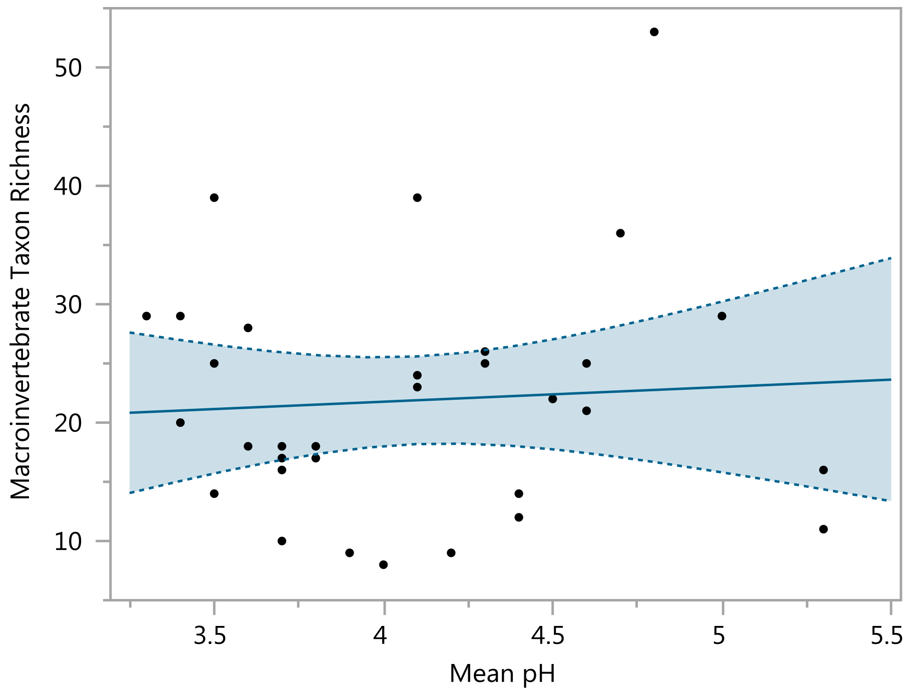
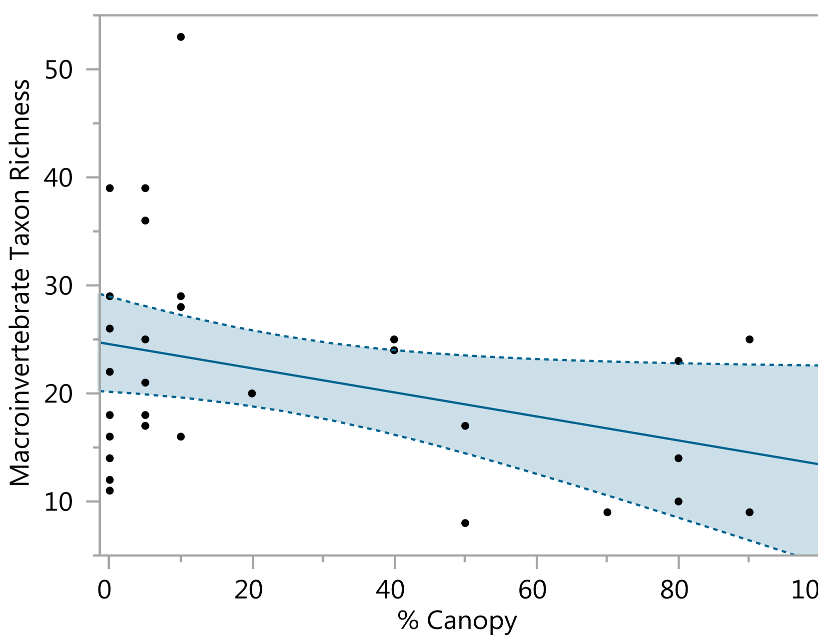


Figure 85. Negative relationship between canopy cover and macroinvertebrate taxon richness (p = 0.051).



COMPARISONS OF AMPHIBIAN COMMUNITIES ACROSS SITE TYPES

Overall, amphibian species richness was highest on enhancement sites, which were slightly higher than open canopy reference sites (Figure 86). Closed canopy reference sites had lower species richness than other site types, most likely because of very low species richness on Block O. Abundance was greater on enhancement sites than all other site types, which were generally comparable. However, quality of species (AQAI) was much higher on open canopy reference sites than all other site types. Enhancement and open canopy reference sites both had higher AQAI than closed canopy reference sites and re-establishment sites, which were not different in this respect.

Anuran species adult body size information was obtained by taking the midpoint of the size range given in Beane et al. (2010) for each species observed (see Appendix E Section: Amphibian Metrics). The smallest anuran species observed was the little grass frog (*Pseudacris ocularis*) (16 mm), which was found on all site types except the closed canopy reference sites (Figure 87). The largest anuran species observed was the bullfrog (*Rana catesbeiana*) (142mm), which was found on all site types, but more often on re-establishment sites. Re-establishment sites generally had larger anurans than other site types, most likely due to the reliable presence of permanent water (Figure 87). Smaller species of anurans were found on the other sites types. The widest size distribution of frog/toad species was found on the open canopy reference sites, but the enhancement sites had the highest total number of amphibian species, which includes salamanders.

In general, amphibian species richness was lowest on closed canopy reference sites and highest on enhancement sites, while abundance varied (Table 36). Block O (a closed canopy reference site) consistently had the fewest amphibian species detected (mean 1.3 species), while Block T (an enhancement site) had the highest number of amphibian species detected (mean 11.7 species). Other sites with high species richness were Juniper Bay (re-establishment sites - mean 10.0 species) and 17 Frog Pond (open canopy reference sites - mean 9.7 species). Mean abundance per dipnet survey was highest by far at Block T (enhancement), and lowest at Block O (closed canopy reference) and Juniper Bay (re-establishment).

Figure 86. Amphibian species richness, mean abundance, AQAI, and mean anuran adult body size by site type (3 years cumulative; no significant change in these parameters occurred among years).

Richness was determined using dipnet/egg mass surveys and frogloggers. Mean abundance was calculated as the mean number of (adult equivalent) amphibians detected during each dipnet and egg mass survey on a site. REFCL = closed canopy reference, RE-ESTAB = re-establishment, REFOP = open canopy reference, ENHANCE = enhancement.

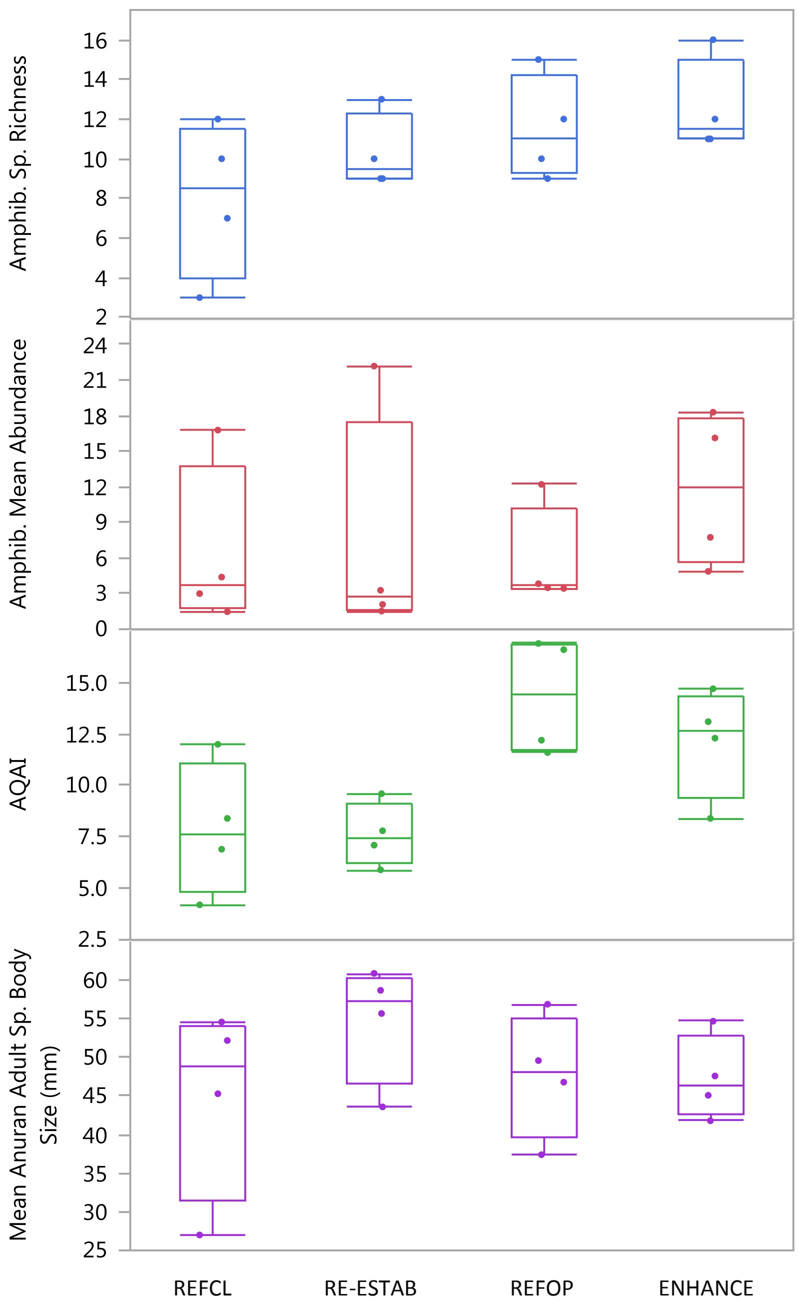


Figure 87. Histograms of adult anuran amphibian species body size (snout-vent length) by site type and year.

REFCL = closed canopy reference, RE-ESTAB = re-establishment, REFOP = open canopy reference, ENHANCE = enhancement.

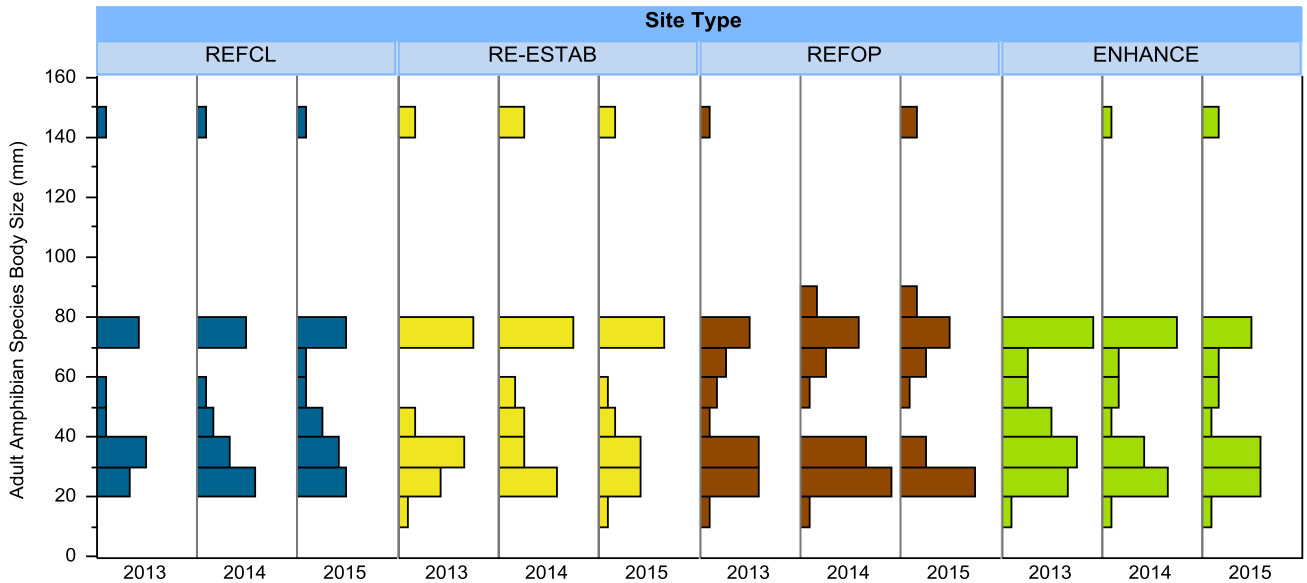


Table 36. Amphibian species richness and mean abundance on each site.

Richness was determined using dipnet/egg mass surveys and frogloggers combined. Mean abundance was calculated as the mean number of (adult equivalent) amphibians detected during each dipnet and egg mass survey on a given site. * denotes sites which had no froglogger data available in 2015 (theft, ants, etc.)

Site Type Code	Site Name	Amphibian Species Richness				Mean Abundance (per dipnet survey per year)
		2013	2014	2015	Mean of All Years	
Closed Canopy Reference	Block O Pond	1	2	1	1.3	0.5
	Cypress Pond	3	3	5	3.7	2.5
	Gum Pond	8	8	9	8.3	9.4
	Pulpwood Pond	8	9	9	8.7	40.2
Re-establishment	Dover Bay	3	8	3*	4.7	26.4
	Juniper Bay	10	9	11	10.0	1.6
	Parker Farms	9	7	2*	6.0	4.8
	Stone Farm	5	3	9	5.7	3.6
Open Canopy Reference	17 Frog Pond	11	11	7	9.7	10.9
	Brandon's Pond	6	11	7	8.0	10.8
	Swain Pond	5	6	8	6.3	5.2
	Tiger Pond	6	7	4	5.7	14.9
Enhancement	Block T Pond	13	11	11	11.7	64.9
	Braswell Ponds	7	6	7	6.7	21.1
	Little Little Dismal Pond	10	8	8	8.7	14.5
	Slate Circle	11	6	2*	6.3	32.7

Table 37. Amphibian species detected on the four site types during the three years of the study. * = NC state listed threatened species

O = observational/dipnet survey, F = froglogger. Species in bold were only detected with frogloggers.

Type of Site	Common Name	Species Name	2013	2014	2015	Total Years
Closed Reference	Southern Cricket Frog	Acris gryllus	OF	OF	OF	3
	Mabee's Salamander	Ambystoma mabeei	O	O	O	3
	Oak Toad	**Bufo quercicus**	F	F		2
	Southern Toad	Bufo terrestris	OF	F	F	3
	Eastern Narrowmouth Toad	Gastrophryne carolinensis	OF	OF	OF	3
	Cope's Gray Treefrog	Hyla chrysoscelis	OF	F	F	3
	Green Treefrog	**Hyla cinerea**			F	1
	Pinewoods Treefrog	Hyla femoralis	OF	OF	OF	3
	Barking Treefrog	**Hyla gratiosa**			F	1
	Squirrel Treefog	Hyla squirella		OF	OF	2
	Eastern Newt	**Notophthalamus viridescens**	O	O	O	3
	Atlantic Coast Slimy Salamander	**Plethodon chlorobryonis**	O			1
	Spring Peeper	Pseudacris crucifer	F	OF	OF	3
	American Bullfrog	Rana catesbeiana	F	O	F	3
	Green Frog	Rana clamitans	OF	OF	OF	3
	Southern Leopard Frog	Rana sphenocephala	OF	OF	OF	3
	Eastern Spadefoot	Scaphiopus holbrookii	O	F	F	3
Re-establishment	Southern Cricket Frog	Acris gryllus	OF	OF	OF	3
	Southern Toad	**Bufo terrestris**	F	F	F	3
	Eastern Narrowmouth Toad	**Gastrophryne carolinensis**	F		F	2
	Green Treefrog	**Hyla cinerea**	F	F	F	3
	Pinewoods Treefrog	Hyla femoralis	OF	O	OF	3
	Squirrel Treefog	Hyla squirella	OF	F	OF	3
	Eastern Newt	**Notophthalamus viridescens**		O		1
	Spring Peeper	**Pseudacris crucifer**	F	F	F	3
	Little Grass Frog	**Pseudacris ocularis**	F		F	2
	American Bullfrog	Rana catesbeiana	F	OF	OF	3
	Green Frog	Rana clamitans	OF	F	OF	3
	Southern Leopard Frog	Rana sphenocephala	OF	OF	OF	3
	Carpenter Frog	**Rana virgatipes**		F	OF	2
Open Reference	Southern Cricket Frog	Acris gryllus	OF	OF	OF	3
	Eastern Tiger Salamander*	**Ambystoma tigrinum**		O		1
	Oak Toad	**Bufo quercicus**	F	F	F	3
	Southern Toad	**Bufo terrestris**	F	F	F	3
	Eastern Narrowmouth Toad	Gastrophryne carolinensis	OF	F		2
	Cope's Gray Treefrog	**Hyla chrysoscelis**	F			1
	Pinewoods Treefrog	Hyla femoralis	OF	OF	F	3
	Barking Treefrog	Hyla gratiosa	OF	OF	OF	3
	Squirrel Treefog	Hyla squirella	F	O		2
	Eastern Newt	**Notophthalamus viridescens**		O	O	2
	Spring Peeper	Pseudacris crucifer		OF	F	2
	Little Grass Frog	**Pseudacris ocularis**	F	F		2
	Ornate Chorus Frog	**Pseudacris ornata**		F		1
	Carolina Gopher Frog*	Rana capito		OF	F	2
	American Bullfrog	**Rana catesbeiana**	F		F	2
	Green Frog	**Rana clamitans**	F	F		2
	Southern Leopard Frog	Rana sphenocephala	OF	OF	OF	3
	Carpenter Frog	**Rana virgatipes**		F		1
	Eastern Spadefoot	Scaphiopus holbrookii	OF		F	2
	Unidentified (egg mass)	Unidentified (egg mass)	O			1

Type of Site	Common Name	Species Name	2013	2014	2015	Total Years
Enhancement	Southern Cricket Frog	Acris gryllus	OF	OF	OF	3
	Eastern Tiger Salamander*	**Ambystoma tigrinum**		O		1
	Oak Toad	**Bufo quercicus**	F		F	2
	Southern Toad	Bufo terrestris	OF	OF	F	3
	Eastern Narrowmouth Toad	Gastrophryne carolinensis	OF	F	OF	3
	Pine Barrens Treefrog	Hyla andersonii	OF			1
	Cope's Gray Treefrog	Hyla chrysoscelis	OF		F	2
	Green Treefrog	**Hyla cinerea**	F	F		2
	Pinewoods Treefrog	Hyla femoralis	OF	OF	OF	3
	Barking Treefrog	Hyla gratiosa	OF	OF	OF	3
	Squirrel Treefog	Hyla squirella	F		O	2
	Eastern Newt	**Notophthalamus viridescens**		O	O	2
	Spring Peeper	Pseudacris crucifer	F	OF	OF	3
	Little Grass Frog	Pseudacris ocularis	OF	OF	O	3
	American Bullfrog	**Rana catesbeiana**		F	F	2
	Green Frog	Rana clamitans	OF	OF	F	3
	Southern Leopard Frog	Rana sphenocephala	OF	OF	OF	3
	Carpenter Frog	**Rana virgatipes**	F	F		2
	Eastern Spadefoot	Scaphiopus holbrookii	OF	F	OF	3

Table 38. Species composition on the four site types, all three years combined.

Species detected only on one site type are in bold. * = NC state listed threatened species

Common Name	Species Name	Closed Reference	Re-establishment	Open Reference	Enhancement
Southern Cricket Frog	*Acris gryllus*	X	X	X	X
Mabee's Salamander	*Ambystoma mabeei*	X			
Eastern Tiger Salamander*	*Ambystoma tigrinum*			X	X
Oak Toad	*Bufo quercicus*	X		X	X
Southern Toad	*Bufo terrestris*	X	X	X	X
Eastern Narrowmouth Toad	*Gastrophryne carolinensis*	X	X	X	X
Pine Barrens Treefrog	*Hyla andersonii*				X
Cope's Gray Treefrog	*Hyla chrysoscelis*	X		X	X
Green Treefrog	*Hyla cinerea*	X	X		X
Pinewoods Treefrog	*Hyla femoralis*	X	X	X	X
Barking Treefrog	*Hyla gratiosa*	X		X	X
Squirrel Treefrog	*Hyla squirella*	X	X	X	X
Red-spotted Newt	*Notophthalamus viridescens*	X	X	X	X
Atlantic Coast Slimy Salamander	*Plethodon chlorobryonis*	X			
Spring Peeper	*Pseudacris crucifer*	X	X	X	X
Little Grass Frog	*Pseudacris ocularis*		X	X	X
Ornate Chorus Frog	*Pseudacris ornata*			X	
Carolina Gopher Frog*	*Rana capito*			X	
American Bullfrog	*Rana catesbeiana*	X	X	X	X
Green Frog	*Rana clamitans*	X	X	X	X
Southern Leopard Frog	*Rana sphenocephala*	X	X	X	X
Carpenter Frog	*Rana virgatipes*		X	X	X
Eastern Spadefoot	*Scaphiopus holbrookii*	X		X	X
Total Number of Species		**17**	**13**	**19**	**19**

Re-establishment sites and closed canopy reference sites were 74% dissimilar in their taxonomic composition and abundance, based on SIMPER analysis (Clarke 1993; Hammer et al. 2001). The Southern Cricket Frog and Southern Leopard Frog were present in much higher numbers on re-establishment sites than reference sites, while the Eastern Spadefoot toad and Eastern Narrowmouth Toad were entirely absent from re-establishment sites but present in higher numbers on the closed canopy reference sites. Many other species including Green Treefrogs, Spring Peepers, Eastern Newts, and Squirrel Treefrogs were present in much higher numbers on closed canopy reference wetlands.

The enhancement sites and open canopy reference sites were 68% dissimilar in their taxonomic composition and abundances, mostly driven by higher numbers of Green Treefrogs, Southern Leopard Frogs, and Eastern Spadefoot Toads on the enhancement sites than the open canopy reference sites. Additionally, Southern Cricket Frogs and Tiger Salamanders were more abundant on the open canopy reference sites. Several additional species were present on the enhancement sites but not on the open canopy reference sites, while one species (Carolina Gopher Frog) was present on open canopy reference sites but not enhancement sites (Table 38).

Two amphibian species listed by North Carolina as state threatened species were detected in this study, the Easter Tiger Salamander and the Carolina Gopher Frog (Table 38). These species were found on the open canopy reference sites and the enhancement sites, but not on the closed canopy reference or re-establishment sites.

Comparison of Highest and Lowest Ranked Sites

Sites were ranked from best to worst based on amphibian species richness and the AQAI (1 being best; 16 being worst) (Table 39). It should be noted that all sites in this study were located in rural areas and surrounded by fairly low levels of human impact, so they do not represent a complete spectrum of best and worst possible in North Carolina. This ranking was done to elucidate variables that may relate to wetland habitat quality in general for amphibians.

Best sites had substantially higher amphibian species richness, AQAI, and slightly higher abundance (Figure 88). Of the 16 sites in this study, sites with the best rankings (rank of 1-5) were open canopy reference sites and enhancement sites. The middle rankings (ranks of 6-11) were found in all site types. The worst rankings (ranks of 12-16) were only re-establishment and closed canopy reference sites.

A closer examination of site rankings and habitat factors can show how sites with a rich amphibian presence differed from those with fewer amphibian species in total or fewer sensitive species. Of the myriad of habitat variables that were measured for this study, we graphed each individually in a preliminary analysis to find those variables that differed markedly between the best and worst ranked sites. Most variables did not differ between the best and worst ranked sites. The results showed that the best sites had lower levels of anthropogenic land use impacts within one mile (LDI) and better water quality (lower specific conductivity and higher dissolved oxygen) (Figure 89). The best sites also had less cover by small shrubs and saplings (<0.5m) and coniferous trees in the wetlands, as well as buffers with less vine cover than the worst ranked sites (Figure 90).

Table 39. Site rankings based on amphibian species richness and quality index (AQAI).

Sites with best rankings have the lowest mean rank number and are darker green, middle rankings are blue, and worst rankings are orange. Lower ranks are better. Sites are sorted by site type: RE-ESTAB = re-establishment sites, REFCL = closed canopy reference sites, REFOP = open canopy reference sites, ENHANCE = enhancement sites.

Site Name	Site Type	Mean Amphib. Sp. Richness	Mean AQAI	Richness Rank	AQAI Rank	Mean Rank
17 Frog Pond	REFOP	9.7	13.5	3	1	2
Block T Pond	ENHANCE	11.7	11.7	1	3	2
Little Little Dismal Pond	ENHANCE	8.7	11.1	5	4	4.5
Pulpwood Pond	REFCL	8.7	11.0	5	5	5
Brandon's Pond	REFOP	8.0	13.3	8.5	2	5.3
Juniper Bay	RE-ESTAB	10.0	7.8	2	9	5.5
Slate Circle	ENHANCE	8.5	9.6	6	7	6.5
Gum Pond	REFCL	8.3	6.6	7	11	9
Tiger Pond	REFOP	5.7	9.8	12.5	6	9.3
Swain Pond	REFOP	6.3	9.5	11	8	9.5
Braswell Ponds	ENHANCE	6.7	7.7	10	10	10
Parker Farms	RE-ESTAB	8.0	5.5	8.5	15	11.8
Stone Farm	RE-ESTAB	5.7	6.4	12.5	12	12.3
Cypress Pond	REFCL	3.7	6.1	15	13	14
Dover Bay	RE-ESTAB	5.5	5.9	14	17	14
Block O Pond	REFCL	1.3	2.5	16	16	16

Figure 88. Amphibian species richness, AQAI, and abundance on sites with lowest and highest rankings.

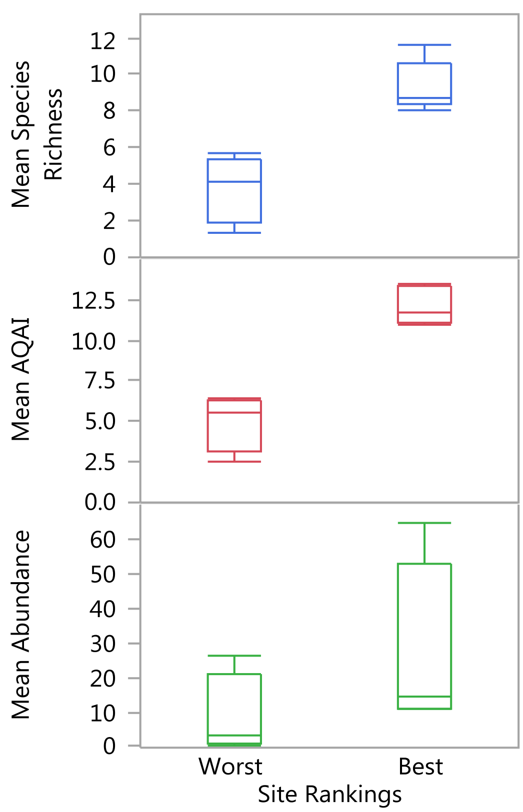


Figure 89. Selected habitat variables with differences between lowest and highest ranked amphibian sites.

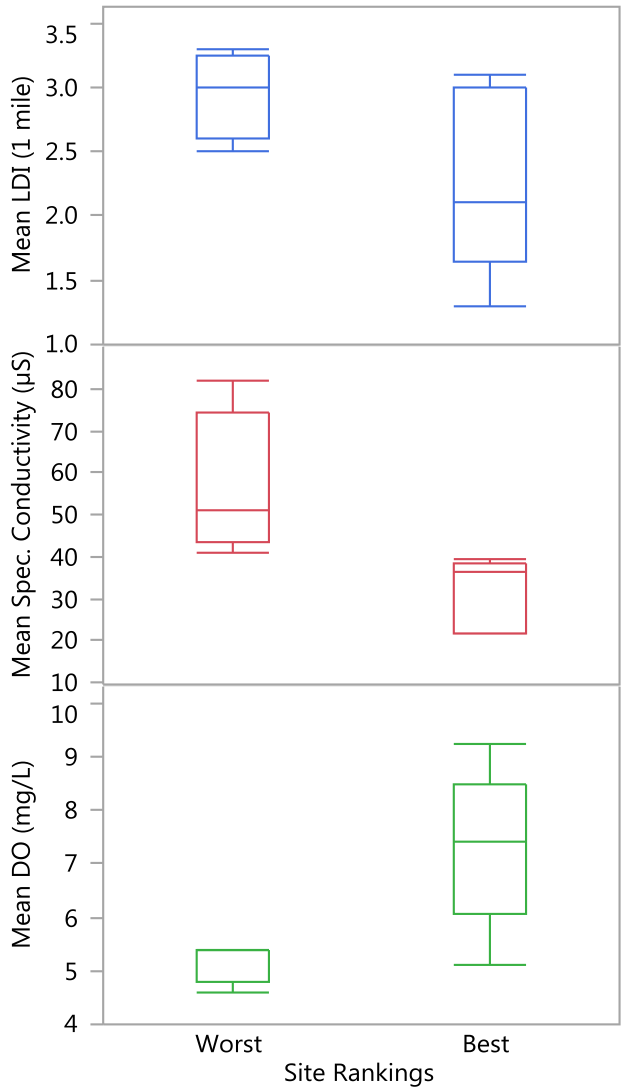
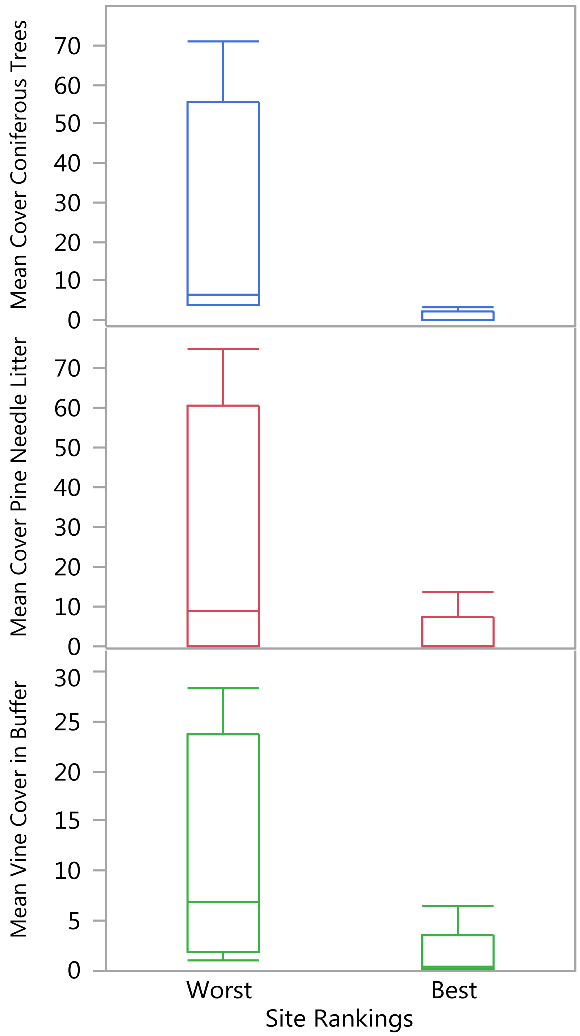


Figure 90. Selected wetland vegetation and buffer variables with differences between worst and best ranked amphibian sites.



Multivariate analyses of factors influencing amphibian communities

A Canonical Correspondence Analysis (CCA) was performed on amphibian communities in a manner similar to that performed on macroinvertebrate communities.

Eighty-four (84) habitat, landscape, and macroinvertebrate variables were correlated with amphibian community metrics using Spearman's non-parametric correlation (Table 40). This resulted in a list of 60 environmental variables that significantly related to the amphibian metrics ($p<0.10$). This list of 60 environmental variables was tested for autocorrelation, to discern which variables highly correlated with each other (Spearman's $r>0.50$ and $p< 0.10$). In the end, 20 variables were chosen for input into the CCA. These 20 variables were each correlated with one or several amphibian metrics and were not highly correlated with each other. Correlations varied in direction and strength (Figure 91).

Table 40. Amphibian metrics tested for correlations with all habitat and landscape variables.

Amphibian Metrics Tested for Correlations	
Species Richness	Amphibian Quality Assessment Index (AQAI)
Abundance	Mean Anuran Species Adult Body Size
Diversity (Shannon's H')	

Cumulative amphibian species occurrence (presence/absence) across the three years of the study was used in the CCA, along with means of environmental variables. The CCA triplot shows how the species occur in relation to the environmental variables with the strongest relationship with the amphibian community (CCA overall p = 0.005; 73.0% of variance explained) (Figure 92). Polygons show how the sites group in relation to the environmental variables. For example, the re-establishment sites were very similar to each other in regards to amphibian community composition as well as having fish, fewer sensitive macroinvertebrate taxa, lower dissolved oxygen, and fewer large trees. The open canopy reference sites had higher dissolved oxygen, no fish, moderate macroinvertebrate taxa richness and sensitive taxa, and few large trees. With the exception of Pulpwood Pond, the closed canopy reference sites occupied the center of the spectrum for all of the important environmental variables. Rankings and the CCA indicated that the best sites for amphibian richness and quality in this study had high dissolved oxygen, very few large trees, and no fish.

Figure 91. Spearman's correlation coefficients showing direction and strength of relationship for the 20 variables that were significantly correlated with amphibian metrics ($p<0.10$) *and* not correlated with each other.

M = macroinvertebrate, ET = Ephemeroptera/Trichoptera, DO = dissolved oxygen, Buff = upland buffer, Fish Pres = fish presence, Shrubs Sapl <0.5m = shrubs and saplings <0.5m height, LDI 500m = Landscape Development Intensity Index (500m around wetland), Dist to Paved Road = distance to nearest paved road, Buff Conif Trees = cover of coniferous trees in upland buffer, Assess Area = size of assessment area or wetland, Veg Sp Richness = dominant vegetation species richness.

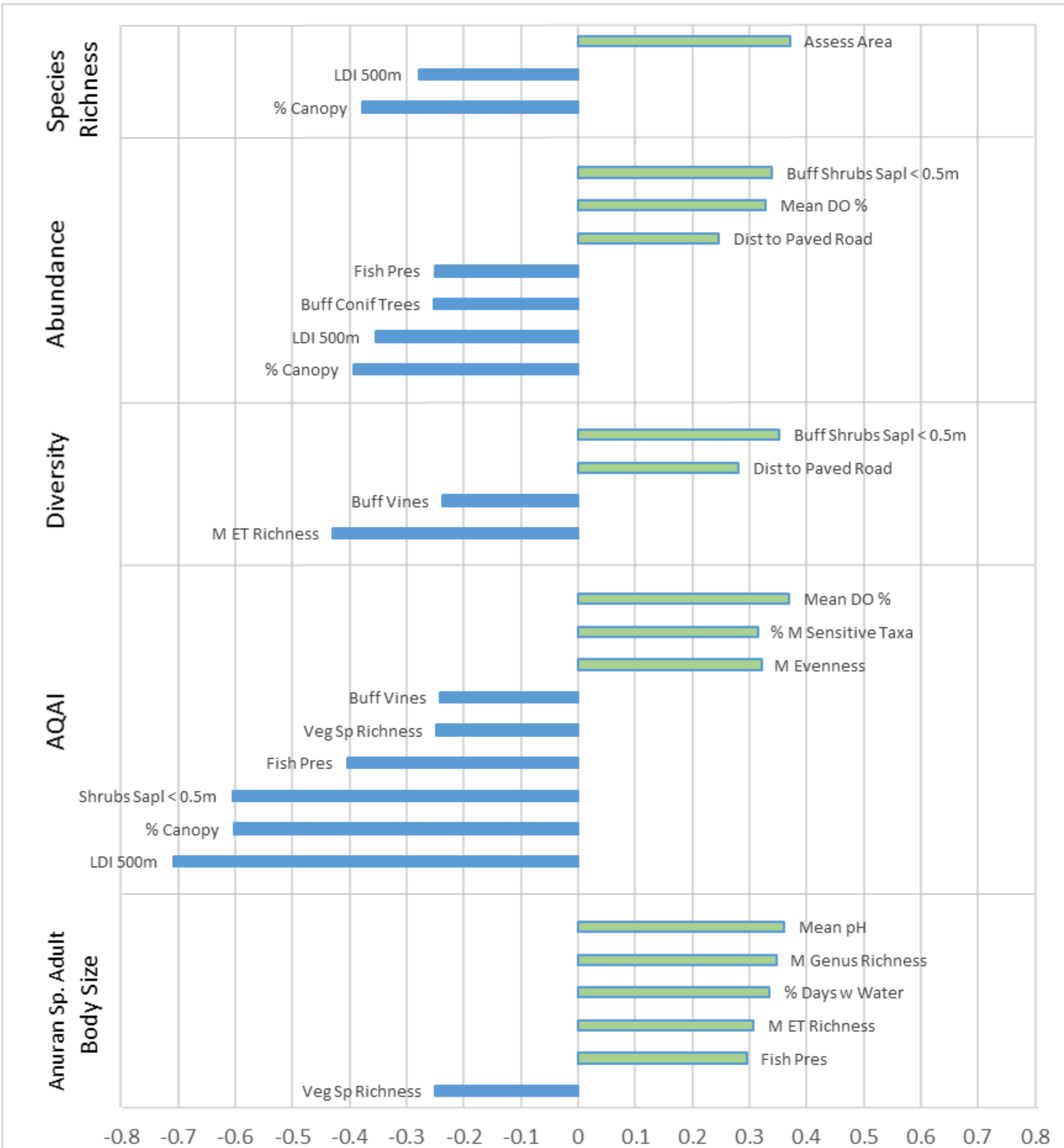
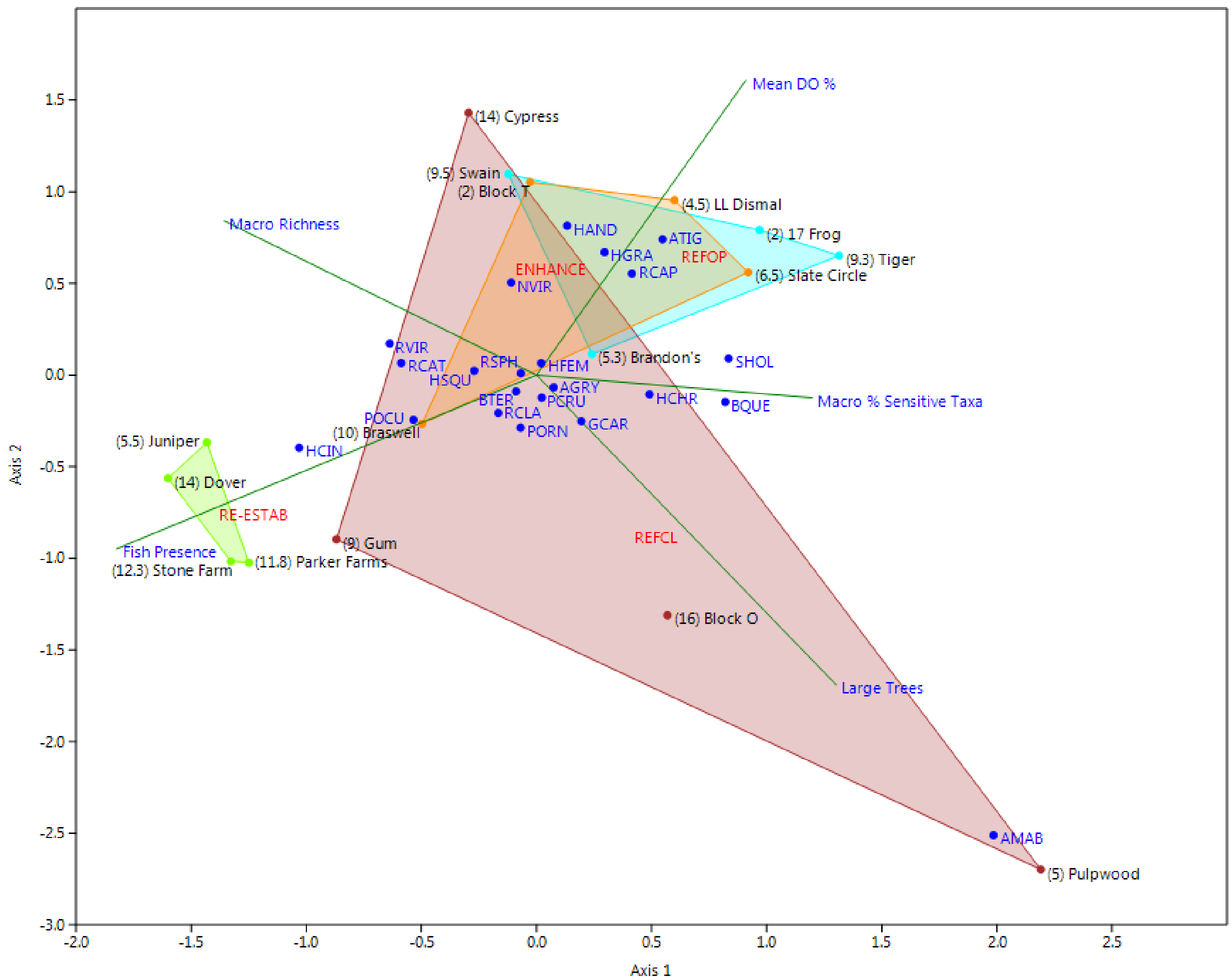


Figure 92. Triplot of Canonical Correspondence Analysis results showing sites, amphibian species, and environmental variables.

Relative length of lines denote strength of influence on amphibian species occurrence. Numbers preceding site names are mean rankings (1=best; 16=worst) based on amphibian species richness and quality index. (CCA overall p = 0.005; 73.0% of variance explained). Sites are grouped by site type. Amphibian species names are represented by a four letter code using the first letter of genus name and first three letters of species name. RE-ESTAB = re-establishment sites, REFCL = closed canopy reference sites, REFOP = open canopy reference sites, ENHANCE = enhancement sites.



Grouping by site location rather than site type seemed to indicate that species composition was more similar among sandhill sites vs. non-sandhill sites, instead of when grouped by site type (Figure 93). Four of the 22 amphibian species (18%) were only found on sandhill sites, so geographic location may have been driving some differences in species composition alongside other factors. These were two salamander species (Mabee's salamander [*Ambystoma mabeei*], Eastern tiger salamander [*Ambystoma tigrinum*]), the pine barrens treefrog (*Hyla andersonii*), and the spadefoot toad (*Scaphiopus holbrookii*). Species in the middle of the triplot were found on both types of sites, but if they were found more often on sandhill sites, they are plotted to the right of center, and vice versa. Higher dissolved oxygen, the largest trees, and the highest proportions of sensitive macroinvertebrate taxa were conditions found in sandhill sites. Fish were present and higher number of macroinvertebrate species were found on non-sandhill sites. All five of these factors were found to be related to species occurrence across the 16 study sites.

Figure 93. Triplot of Canonical Correspondence Analysis results showing sites, amphibian species, and environmental variables in the sandhills region vs. non-sandhills region.

Relative length of lines denote strength of influence on amphibian species occurrence. Sites are grouped by location within North Carolina (sandhills region vs. non-sandhills region). (CCA overall p = 0.005; 73.0% of variance explained). Amphibian species names are represented by a four letter code using the first letter of genus name and first three letters of species name. RE-ESTAB = re-establishment sites, REFCL = closed canopy reference sites, REFOP = open canopy reference sites, ENHANCE = enhancement sites.

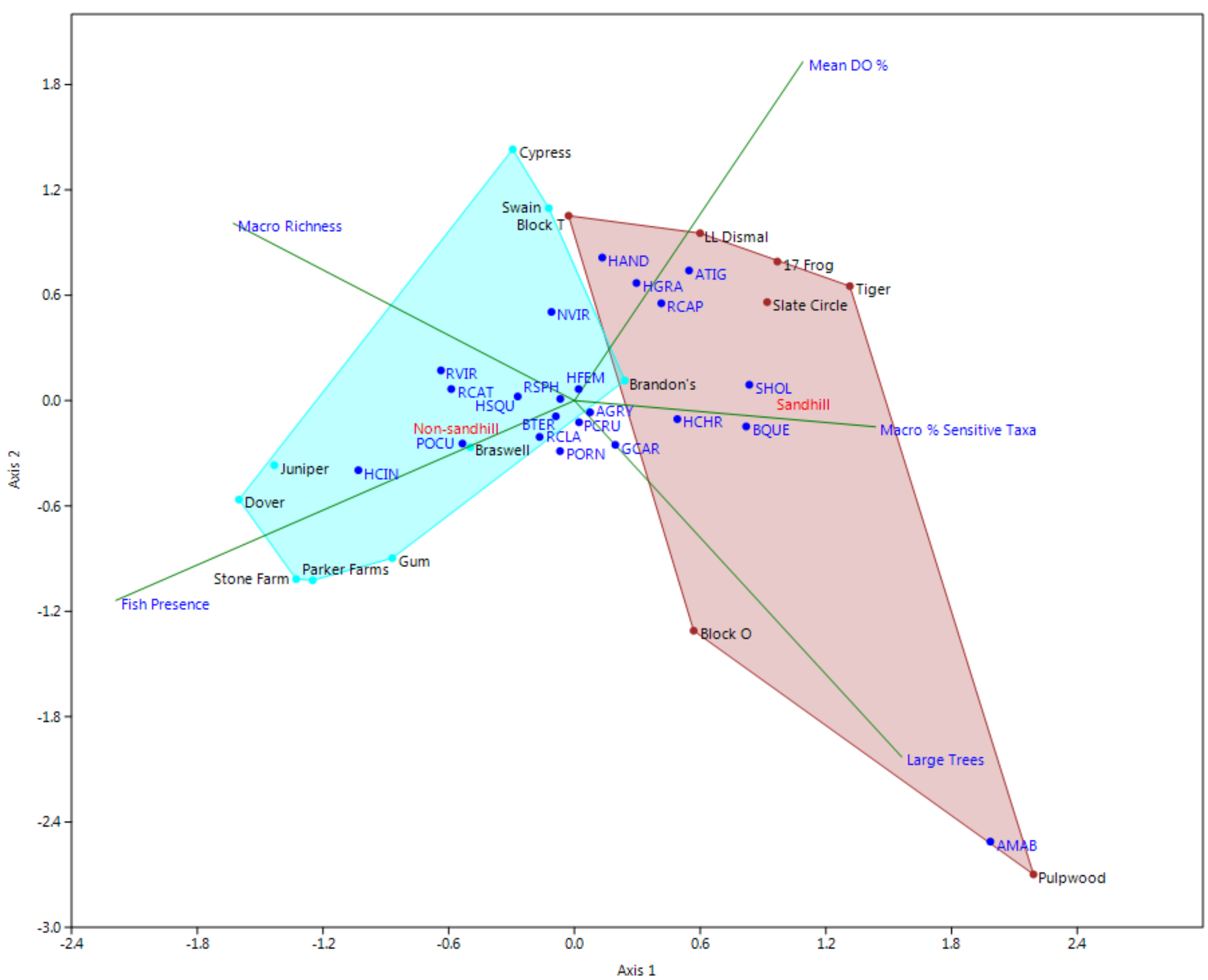
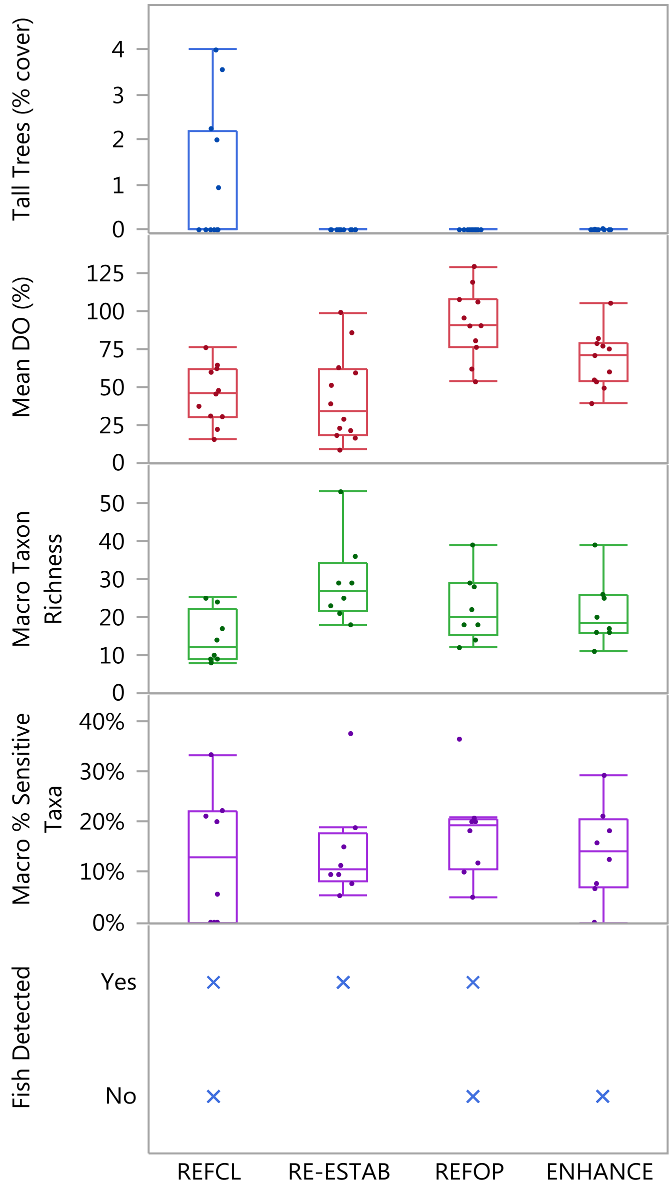


Figure 94. Box plots by site type of the five variables found to have important influence on amphibian communities based on the Canonical Correspondence Analysis.



In comparison to closed canopy reference sites, re-establishment sites had significantly lower cover of tall trees (Wilcoxon test, p=0.076) (Figure 94). Re-establishment sites had significantly higher macroinvertebrate taxon richness (Wilcoxon test, p=.009), and fish were present at all sites (sometimes present on closed canopy reference sites). No difference was detected in mean percent sensitive macroinvertebrate taxa or dissolved oxygen (Wilcoxon test, p>0.10).

In comparison to open canopy reference sites, enhancement sites had lower dissolved oxygen (Wilcoxon test, p=0.015) and fish were never present (Wilcoxon test, p=0.078). Open canopy sites and enhancement sites were no different in terms of tall trees, macroinvertebrate taxon richness, and percent sensitive macroinvertebrate taxa (Wilcoxon test, p>0.10).

COMPARISON OF AMPHIBIAN FINDINGS TO LITERATURE

Relationships Between Amphibian Species Occurrence and Environmental Parameters

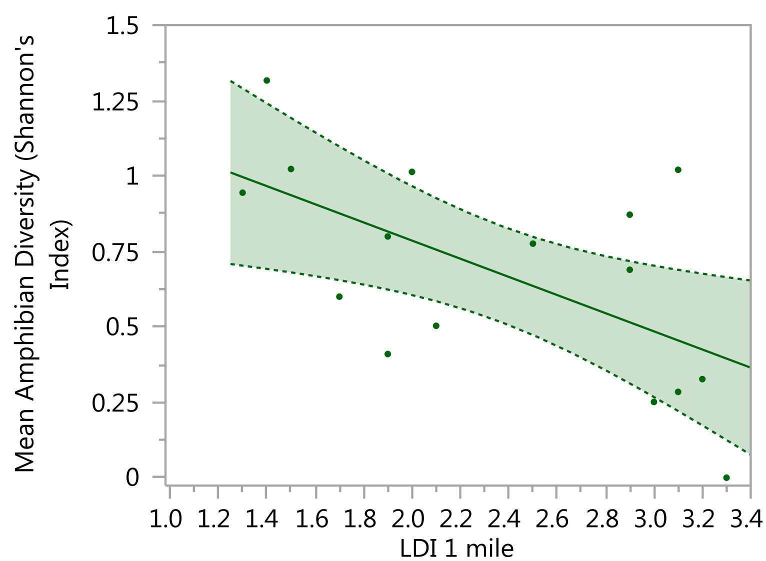
Landscape setting and buffers:

Terrestrial habitat surrounding wetlands is of tremendous importance to amphibians, as most species utilize wetlands for reproduction and retreat to upland habitats for cover and food during intervening times. The continued persistence of amphibian populations depends on available upland habitat just as much as wetland habitat (Gibbons 2003).

Effects of land use on amphibian species richness and abundance peak around 2000 to 3000 meters from a wetland (Findlay and Houlahan 1997; Hecnar and M'Closkey 1998; Houlahan and Findlay 2003). Additionally, road density is related to lower amphibian species richness and abundance (Houlahan and Findlay 2003). Amphibian species diversity in our study was significantly negatively correlated with higher levels of development within one mile of the wetlands (Spearman's p=0.012) (Figure 95). Increasing distances from roads were also associated with significantly higher species richness and better AQAI values (Figure 96).

Distance to source wetlands has been found to be an important factor in predicting amphibian species richness in natural wetlands (Guzy 2010) and recently restored wetlands (Lehtinen and Glatowitsch 2001). We found no relationship between mean distance to nearest two (amphibian accessible) wetlands and any amphibian metric, possibly because all wetland sites were located in rural, generally undeveloped settings.

Figure 95. Significant negative relationship between amphibian species diversity and landscape development intensity index within one mile (LDI 1 mile) (Spearman's p=0.012).



Small wetlands are just as important as large ones in maintaining the long-term persistence of amphibian species. Studies in Georgia, Florida, Washington, and Canada have found no relationship between wetland size and species richness in natural wetlands (Snodgrass et al. 2000, Gonzalez 2004, Richter and Azous 1995, Hecnar and M'Closkey 1996; Lehtinen and Galatowitsch 2001). Our study showed a positive correlation between wetland size and species richness (when re-establishment sites were excluded) (Spearman's p = 0.091), but removal of a particularly large and species rich wetland (Block T) caused the relationship to no longer be statistically significant (Spearman's p = 0.273) (Figure 97). In contrast, Lehtinen and Galatowitsch (2001) found that wetland size only correlated with higher species richness in restored wetlands, and not in reference wetlands. Houlahan and Finlay (2003) found amphibian species richness to be (weakly) positively correlated with wetland area.

Figure 96. Significant positive relationships between distance to nearest road and AQAI (Spearman's p=0.022) and amphibian species richness (Spearman's p=0.086).

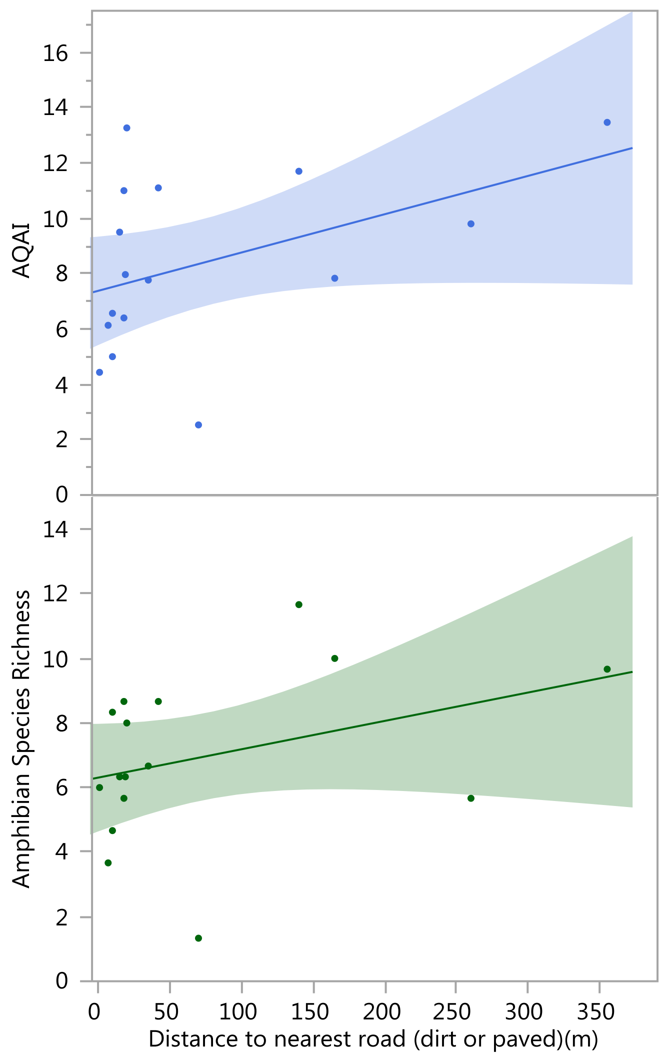
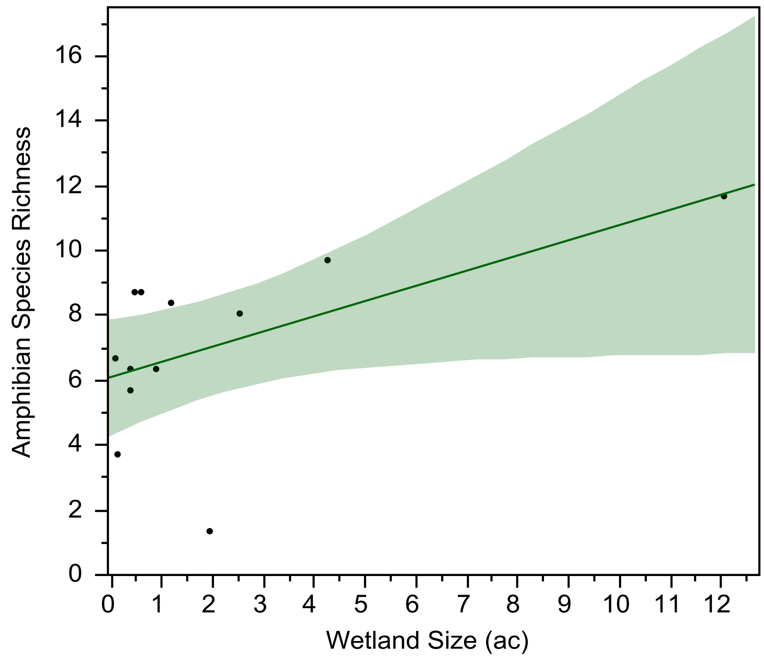


Figure 97. Relationship between amphibian species richness and wetland size (re-establishment sites excluded).



Water Quality:

Mean specific conductivity was lower on closed canopy reference sites than re-establishment sites, and lower on open canopy reference sites than enhancement sites (Figure 98). Overall amphibian abundance and quality were both significantly negatively correlated with specific conductivity (Figures 99), similar to the results of Browne and Paszkowski (2009). Amphibian diversity and richness were not correlated with specific conductivity in our study. However, Babbitt et al. (2006) found greater species diversity with lower specific conductivity in Florida marshes within an agricultural setting, where mean conductivity (94.9 µS) was much higher than in our study (mean 46.2 µS).

Mean dissolved oxygen was highest in the open canopy reference wetlands and lowest on the re-establishment sites (Figure 98). Browne and Paszkowski (2009) found amphibian abundance to be positively related to dissolved oxygen.

Mean water temperature was similar across all site types.

Figure 98. Comparison of various water quality measurements by site type.

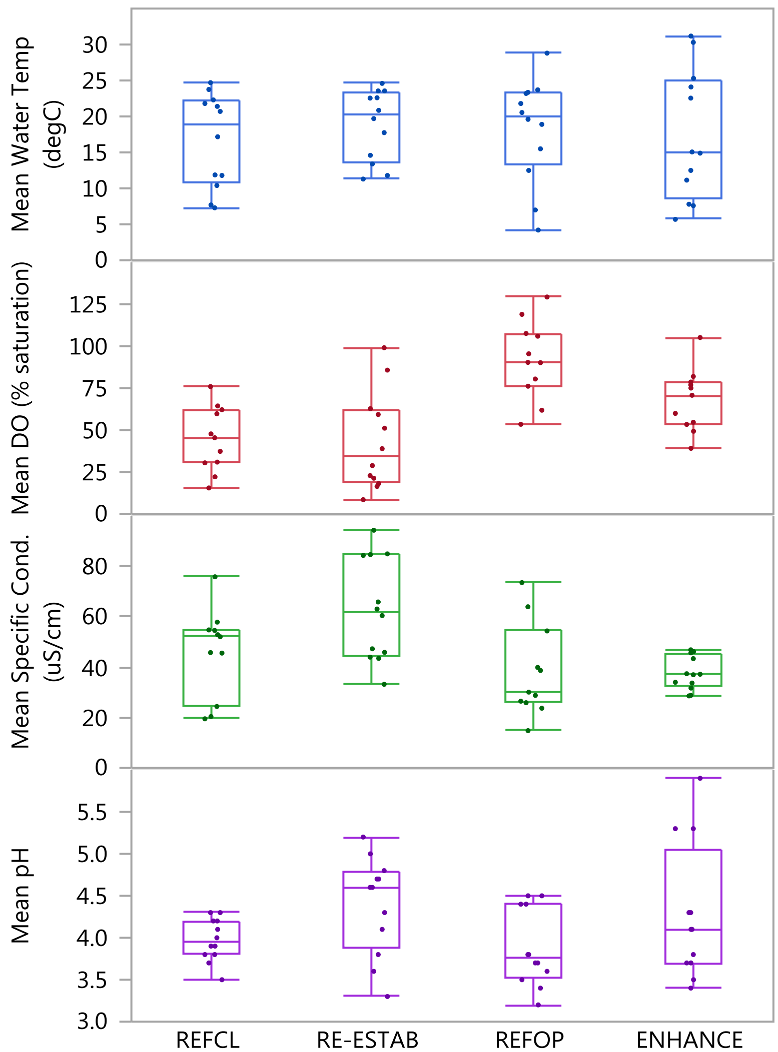
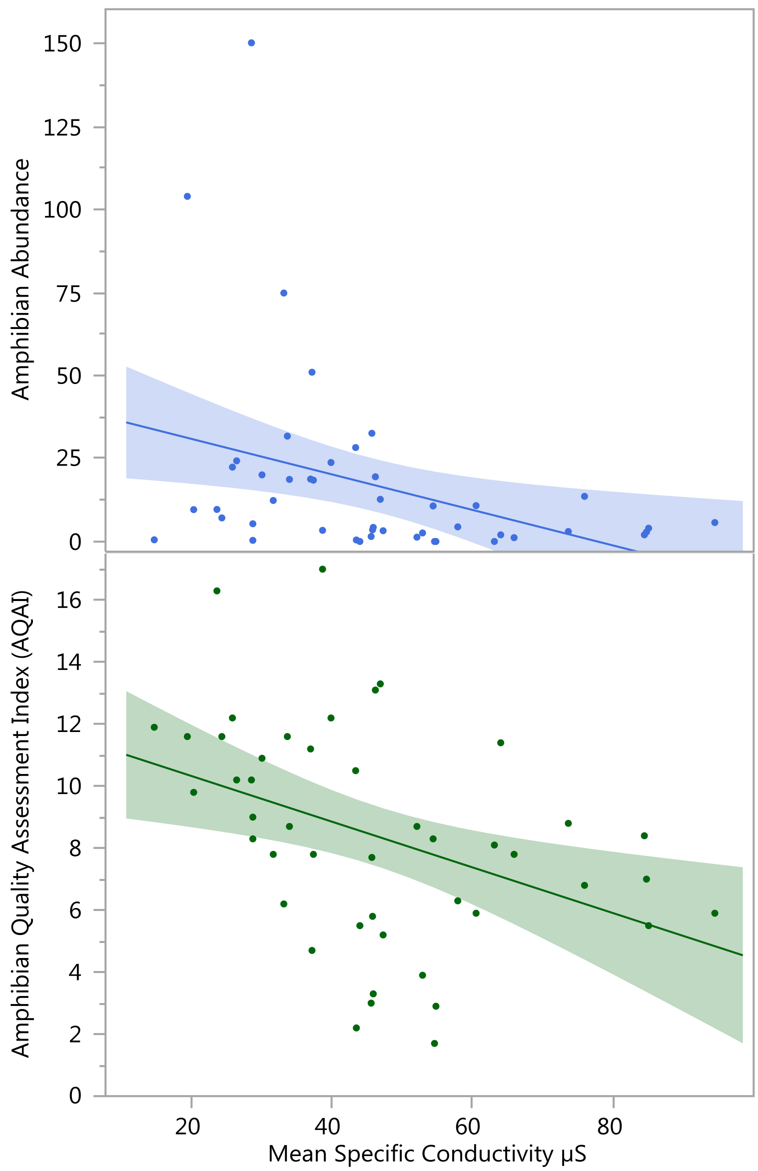


Figure 99. Significant negative relationships between specific conductivity and amphibian abundance (p=0.013) and AQAI (p=0.004).



The range of mean pH values found in the wetlands in this study was low, from 3.2 to 5.9 (Figure 100). The highest pH values were found on the re-establishment sites (except Stone Farm) and Slate Circle (an enhancement site). Slate Circle may have historically received soil amendments during its agricultural use. The lowest pH values were found on a combination of open canopy reference, closed canopy reference, enhancement, and one re-establishment site (Stone Farm). In general, mean pH was lower on closed canopy reference sites than re-establishment sites, and lower on open canopy reference sites than enhancement sites (Figure 100).

Smith and Braswell (1994) studied the occurrence of four amphibian species in North Carolina in relation to pH of wetlands they were found to breed in. They found closed canopy depressional wetlands to have significantly lower pH than open canopy wetlands. Though our sample size is small, pH in our open canopy reference wetlands was lower than closed canopy reference wetlands; all were located within pine flatwoods communities. Enhancement sites had higher mean pH than open canopy reference sites, suggesting that recent fire and clearing had the effect of raising the pH by reducing the buildup of peat. Mean pH on re-establishment sites was higher than on all other site types.

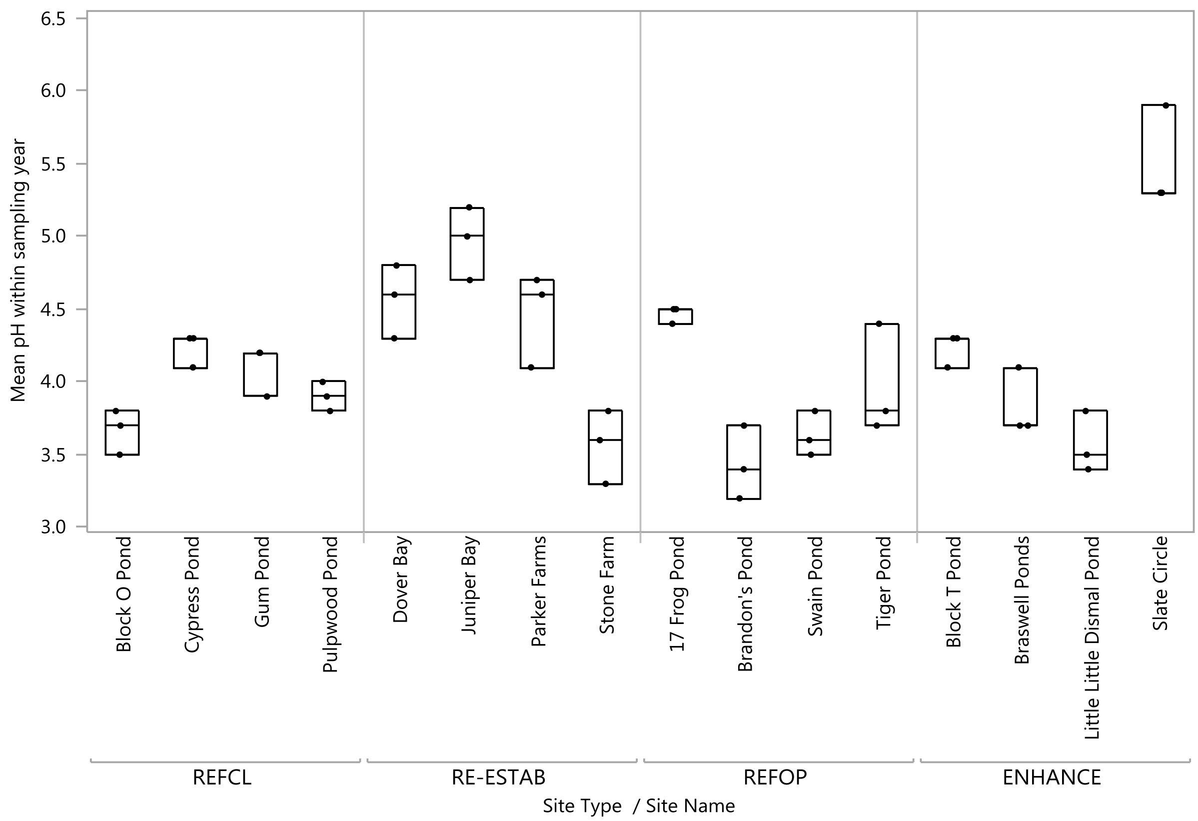
Smith and Braswell (1994) concluded that the Carolina gopher frog (*Rana capito*) and Mabee's salamander (*Ambystoma mabeei*) prefer sites with a relatively high pH values (4.2 – 5.2 for *R. capito*; 4.3 – 6.6 for *A. mabeei*). Though the Carolina gopher frog was rare (3/16 sites) in our study, adults, eggs, and larvae were found in wetlands at lower pH levels (3.4 – 4.5) than in Smith and Braswell (1994). Mabee's salamander (adult and larvae) was encountered at one closed canopy reference site (Pulpwood Pond), which had a mean pH of 3.8 to 4.0 over three years. This pH level is also below that reported by Smith and Braswell (1994) for Mabee's salamander (4.3 – 6.6). However, Fairman et al. (2013) reported a related salamander, the marbled salamanders (*Ambystoma opacum*), was found more frequently in the less acidic sites in a sample of acidic ponds (pH 3.36 – 4.41).

Clark (1986) found decreased abundance of adults and egg masses at lower pHs in Ontario (range 4.44 to 6.63), although species specific differences did exist, i.e. some species were abundant at the lowest pH (wood frogs, green frogs) and some were not found at pH lower than 5.0 (bullfrog and American toad). Low pH (lower than 4.2) can have a detrimental effect on hatching success and survival of larval amphibians (Sadinski and Dunson 1992).

We found no relationship between pH and amphibian species richness in our study, but the lack of an observed relationship may have been affected by the restricted range of pH values in our study wetlands (3.2 – 5.9). In another North Carolina study, amphibian species diversity was lower in ephemeral depressions with low pH (below 4.5) (Smith and Braswell 1994). Gonzalez (2004) found no correlation between amphibian species richness and water pH in 12 wetlands over 2 years, but Babbitt et al. (2006) found higher amphibian species richness at more acidic pH values (pH range in their study was 4.5 – 7.1). In all likelihood, a pH range exists that allows for optimal amphibian species richness, and this optimal range appears to be somewhat acidic. Ranges of pH values in study wetlands are sometimes unreported, so it is possible that studies reporting an increased richness with increasing pH are studying the lower pH values or left side of an optimum curve, and studies reporting a decreased richness with increasing pH are studying the higher ranges of optimal pH values.

Figure 100. Mean pH by site.

Dots represent mean pH for a given year of the study.



Hydroperiod:

In a review of research on amphibians in re-establishment and reference wetlands, Brown et al. (2012) found that an intermediate hydroperiod fostered the highest amphibian species diversity, because it allows most amphibian taxa to complete larval development while also keeping the wetlands fish free. We found a similar positive association, where a curvilinear relationship fit the data better than a linear one and suggested a longer, but not permanent, hydroperiod resulted in the highest species richness (Figure 101). Snodgrass et al. (2000) found a similar pattern where amphibian species richness peaked at a hydroperiod of 8-10 months. Several other investigators have found a positive correlation between amphibian species richness and hydroperiod length (Pechmann et al. 1989, Semlitsch et al. 1996, Nuzzo and Mierzwa 2000, Babbitt et al. 2004, Henning 2004, Guzy 2010).

In studies of amphibians in southeastern US wetlands, Snodgrass et al. (2000) found many species adapted to ephemeral wetlands were not found in wetlands with long hydroperiods. This same pattern was observed in North Dakota (Euliss and Mushet 2004). Snodgrass et al. (2000) concluded that in addition to longer hydroperiod wetlands, shorter hydroperiod wetlands are essential to maintaining biodiversity of amphibians across a landscape, because they support species not found in longer hydroperiod wetlands.

In our study, most amphibian species were present at both ephemeral wetlands and long hydroperiod wetlands (Table 41). It should be noted, however, that several long hydroperiod sites were also fish free, and fish absence was shown to be an important factor influencing amphibian community composition in our study (Figure 92) and others (Guzy 2010; Balcombe et al. 2005b). Southern cricket frogs (*Acris gryllus*), barking treefrogs (*Hyla gratiosa*), and the ornate chorus frog (*Pseudacris ornata*) have been found less frequently in wetlands with fish (Liner 2006). Babbitt et al. (2004) also found amphibian species composition shifts in response to the presence of fish. Monitoring efforts to evaluate isolated and/or re-establishment wetlands should take into consideration the effect of fish on amphibian species, before drawing conclusions based solely on amphibian surveys.

Figure 101. Curvilinear relationship between hydroperiod length and amphibian species richness (p=0.056).

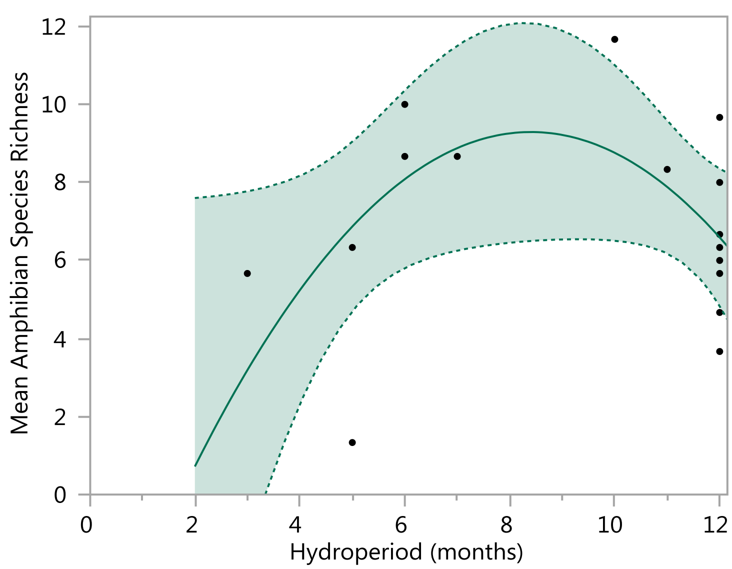


Table 41. Amphibian species presence by hydroperiod length (months) in fish free wetlands and wetlands with fish.

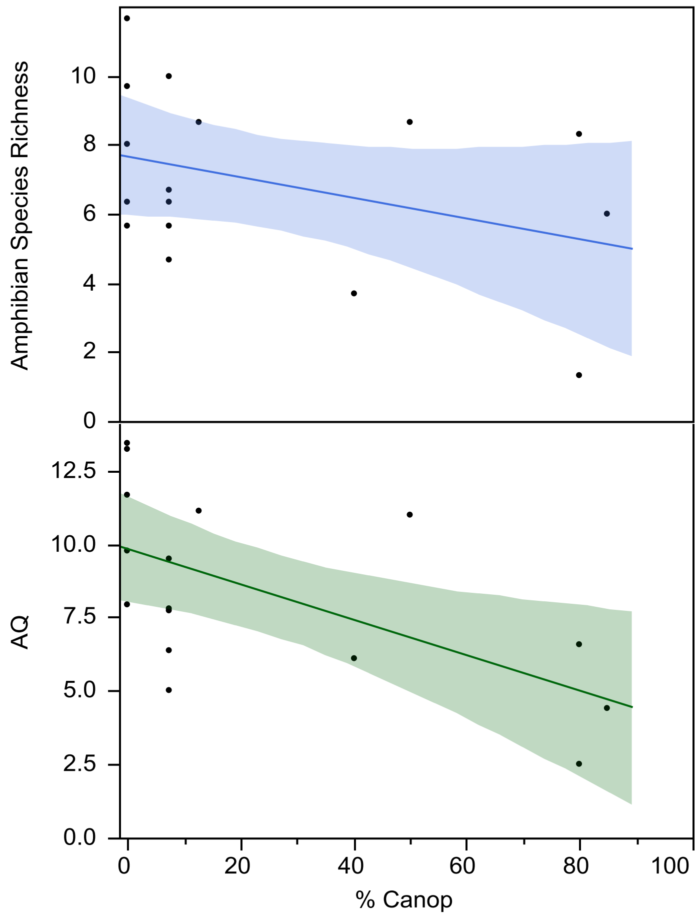
Four letter species code is first letter of genus and first three letters of species name. “X” denotes species detection during the three-year study.

Species Code	Hydroperiod Length (months) Fish Free Wetlands										Hydroperiod Length (months) Wetlands with Fish						
	3	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
AGRY	X		X	X	X			X		X	X					X	X
BTER	X		X	X	X			X		X	X					X	X
GCAR	X		X	X	X			X		X	X					X	X
HFEM	X		X	X	X			X		X	X					X	X
PCRU	X		X	X	X			X		X	X					X	X
RSPH	X		X	X	X			X		X	X						X
RCLA			X	X	X			X		X	X					X	X
HGRA			X		X			X		X							X
NVIR	X				X			X		X	X						
SHOL	X		X	X				X		X							
HCHR			X	X				X		X						X	
ATIG	X		X					X		X							
RCAT								X		X	X					X	X
RVIR								X		X	X						X
BQUE	X			X	X					X							X
HSQU			X							X	X					X	X
POCU										X	X						X
RCAP										X							X
HAND					X			X									
HCIN								X			X					X	X
AMAB				X													
PORN																	X

Fire and canopy cover:

Amphibian richness and abundance have been found to be positively correlated with emergent vegetation cover (Brown et al. 2012) and negatively associated with canopy cover (Skelly et al. 2005). We found higher amphibian species richness and quality in open canopy systems (Figure 102). Removal of trees in historically open-canopy systems (by burning or selective cutting) can benefit amphibians (Thurgate and Pechmann 2007). Amphibian diversity was higher in (routinely burned) pine forests than in hardwood forests surrounding Carolina bay wetlands in South Carolina (Hanlin et al. 2000).

Figure 102. Negative relationships between percent canopy cover and amphibian species richness and Amphibian Quality Assessment Index (AQAI).



Amphibian Communities and Wetland Re-establishment

Balcombe et al. (2005b) did comprehensive ranking of re-establishment and reference sites using vegetation, invertebrates, birds, and anurans. They observed lower plant diversity in reference wetlands and higher plant diversity in re-establishment wetlands. Wetlands with good amphibian rankings in their study were larger and more heterogeneous, without fish, and contained equal ratios of emergent vegetation to open water. Additionally, Porej and Hetherington(2005) and Shulse et al. (2012) found fish free re-establishment sites with shallow wetland edges to be associated with much higher amphibian species richness than those without these features. In our study, wetlands with the best amphibian rankings had better water quality, more aquatic plants, and fewer pine trees and small shrubs.

Brown et al. (2012) did a meta-analysis of 37 studies on re-establishment wetlands and amphibians (in NC and around the world), and found that in nearly all of the reviewed studies, amphibian abundance and richness at created and enhanced wetlands was similar to or greater than reference wetlands. We found generally higher amphibian species richness at re-establishment sites than closed canopy reference sites, perhaps because re-establishment sites were in transition from more open systems to closed systems, as well as containing more permanent water. Amphibian abundance and size of the anuran species were similar between re-establishment and closed canopy reference sites. However, closed canopy reference sites provided habitat for sensitive amphibian species more often than re-establishment sites did (higher maximum AQAI) (Figure 86). Our results confirm Brown et al.'s conclusion that "creating and enhancing wetlands can be valuable tools for amphibian conservation. However, the ecological needs and preferences of target species must be considered to maximize the potential for successful colonization and long-term persistence." Some have concluded that amphibian biodiversity in forested and shrub wetlands is not being replaced by constructed re-establishment wetlands (Porej 2004; Micacchion 2004).

Lehtinen and Galatowitsch (2001) found recently enhanced wetlands to recruit a subset of amphibian species that occurred in natural reference wetlands. We found some overlap in the species composition between re-establishment sites and closed canopy reference sites; however, a few species were unique to each type of wetland (Table 38) and the two state-listed threatened species found in this study (tiger salamander [*Ambystoma tigrinum*] and Carolina gopher frog [*Rana capito*]) were only found on open canopy wetlands. Eleven (11) species were found in common between the re-establishment sites and closed canopy reference sites in our study; six species were found on closed canopy wetlands and not on re-establishment sites (Mabee's salamander, Oak toad, Cope's Gray Treefrog, Barking Treefrog, Atlantic Coast Slimy Salamander, and Eastern Spadefoot Toad), and two species were found on re-establishment sites that were not found on closed canopy reference sites (Little Grass Frog and Carpenter Frog). All in all, 17 species were found on closed canopy sites, and 13 species were found on re-establishment sites (19 on both open canopy reference and enhancement sites).

Discussion and Recommendations

RE-ESTABLISHMENT AND AQUATIC FAUNA

Pond-breeding amphibians are an important part of overall wetland ecosystems as essential links between lower and higher trophic levels, and continued wetland losses in the Southeast are of major concern to the long-term persistence of amphibians. Re-establishment through wetland enhancement or restoration is done in an effort to replace lost wetland acreage and function. It is therefore critical that re-establishment and conservation efforts be managed in a way that increases both wetland and upland amphibian habitat.

In North Carolina currently, most mitigation is done through the state's In Lieu Fee program, which maintains large mitigation sites. These large re-establishment sites may function to provide breeding habitat for amphibian metapopulations or patchy populations if they also provide strong habitat heterogeneity, e.g. providing areas that are not all hydrologically connected, providing for the possibility of fish free breeding areas, areas with longer and shorter seasonal hydroperiods, and wide adjacent upland buffers for non-breeding amphibians. However, replacement of amphibian populations that are lost to development is only possible if re-establishment is done in close proximity to other wetlands. Mitigation sites should be located near natural wetlands which can serve as sources for wetland dependent fauna and flora. Incorporating these factors into mitigation design will help accomplish the goal of creating self-sustaining systems that enable long-term persistence of resident populations. Maintenance or creation of intact forested connections between wetlands should be a high priority, since many amphibian species avoid open areas and forest edges (Regosin et al. 2005). Other research shows that protection of only a narrow strip (15 – 30m wide) of suitable upland surrounding a wetland is insufficient for maintenance of amphibian populations (Regosin et al. 2005; Semlitsch and Bodie 2003). A minimum protected upland buffer of 200 meters is recommended (Regosin et al. 2005). Clearing within 200 meters of a breeding pond impacts a large proportion of the local amphibian population, since a majority have been found within 290 meters away from a wetland during the non-breeding season (Regosin et al. 2005; Semlitsch and Bodie 2003).

Many of our natural reference sites dried out regularly, if not every year, which eliminated fish and increased habitat suitability for larval amphibians. Mitigation design should include efforts to increase habitat heterogeneity within wetlands, and include areas with differing hydroperiods. Mitigation wetlands that go through wet and dry cycles more closely resemble and function like natural wetlands (ELI 2004). That said, hydrology exceeding the minimum requirement of 12.5% consecutive days during the growing season within 12” of surface would be needed for amphibians ponds to provide appropriate habitat. The best ranked sites for amphibians had between 35% and 100% hydrology (Tables 32 and 39). The best ranked sites for macroinvertebrates had between 75% and 100% hydrology (Tables 32 and 33).

Small, disconnected wetlands are important sources of biodiversity, and attention should be given to their mitigation (Gibbs 1993; Kirkman et al. 1999). Unfortunately, in North Carolina there is no financial or regulatory incentive to use small isolated wetlands or small non-isolated wetlands that do not have a hydrological connection that would allow fish to enter. This is in part because the USACE does not require mitigation for isolated wetland impacts, and even though NC DWR does, mitigation providers have financial incentive to satisfy both agencies with one site. Because compensatory mitigation is financially driven, and solvency is a priority, most mitigation providers try to acquire large enough project sites to avoid a financial loss. North Carolina does not currently have instruments in place to incentivize mitigation or preservation of small isolated wetlands or wetland complexes for mitigation. That said, a few examples do exist in the state, where mitigation banks were created around a series of isolated basins and pocosins. However, long-term stewardship and maintenance remain uncertain.

To maximize habitat potential for amphibians, large mitigation sites should be designed to mimic the natural mosaic of wetlands that vary greatly in size, hydroperiod, canopy cover, fish presence, and connectedness, with undeveloped upland connections between them. Amphibians benefit from a complex of habitats that include wetlands (Knutson et al. 1999). A mosaic of upland and wetland areas would more closely mimic natural systems and provide amphibians with the diverse array of breeding habitat conditions they need for long term persistence, and subsequently provide important feeding opportunities for other vertebrates. Mitigation sites may be subject to site-wide extinctions due to environmental perturbations such as drought, disease, and fish presence, particularly if they do not provide a complex array of habitat conditions (Petranka et al. 2007). Also, wetland diversity within a landscape is vital because no individual wetland contained all species sampled (Table 36). Analysis of the differences between the best and worst ranked sites showed that macroinvertebrates and amphibians thrive under different sets of habitat conditions; therefore building diverse microhabitats and heterogeneity into mitigation design is critical to restoring habitat for both macroinvertebrates and amphibians.

Within a wetland, most of our open canopy reference sites and enhancement sites had a deeper area that often didn't dry out completely, even in the summer. Most of the pool areas contracted and expanded with changing conditions, but maintained a core wet area available to some aquatic fauna. Mitigation sites could be designed with this natural topography in mind, where water is shallow enough to dry out and keep fish out, except for a limited "reserve" area where it is deeper.

Our results show that effective wetland restoration (compensatory or non compensatory) will not be accomplished with narrow buffer zones around wetland areas. Mitigation sites need to include protection of adequate upland buffers as habitat to ensure full function of wetlands for amphibian populations. In North Carolina, upland does not generate mitigation credit, minimal incentive exists to preserve upland buffers as part of a larger mitigation site. Upland currently represents a liability with no return on investment. However, stream mitigation projects do require upland buffers to be associated with restored streams (50 feet in the Piedmont and Coastal Plain; 30 feet in the Mountains). North Carolina is considering providing extra stream mitigation credit for buffers up to 200 feet from a restored stream, and new buffer rules will provide extra credit for buffers or nutrient offset for up to 200 feet from streams. There is potential for small wetlands to exist within 200 feet of a stream and provide habitat for amphibians, especially if the stream is intermittent or a small perennial stream. This recent incentive for extra buffer, nutrient, or stream habitat may help include some amphibian habitat. Depressions within a stream buffer may or may not be fish free, especially with large streams, so some amphibian species may be excluded from this type of breeding opportunity.

At the present time, the task of preserving, restoring, and maintaining small, disconnected wetlands may be best performed by land trusts or public land managers (such as NC WRC, for example). Long term stewardship is an important consideration in the maintaining viability of wetlands for aquatic biota. For North Carolina mitigation sites, the only stewardship required is monitoring of the conservation easement boundary on the property. The state has taken that on for the In Lieu fee program, but currently only designates two employees for monitoring easement integrity of a large number of mitigation sites across the state. In many other states, a mitigation banker must have funds up-front for long term site management, but North Carolina does not require this. The NC DOT monitors the easements on their own sites, as do private mitigation bankers. In addition, merely assuring the integrity of a conservation easement boundary is not necessarily sufficient as long term stewardship of a wetland site. Many of North Carolina's wetlands were historically part of fire-dependent ecosystems, and controlled burning should be part of a long-term management strategy to maintain amphibian habitat. This is not being done in North Carolina, because for most sites there is no funding for active long-term management of mitigation sites.

MONITORING AMPHIBIANS ON MITIGATION SITES:

Very few states currently incorporate measures of any kind of biotic communities into their wetland mitigation success criteria; hydrology tends to outweigh biotic performance. When they exist, the vast majority of biotic-based performance standards for compensatory mitigation revolve around vegetation, including dominance by native species, minimal coverage of non-native species and bare ground, minimum surviving stem counts for native woody species (forested mitigation), numbers of native plant species, floristic quality index values, etc. (ELI 2004). However, measures focused on aquatic fauna are needed to truly assess whether mitigation is accomplishing the task of replacing lost habitat function.

Freshwater wetland mitigation success criteria in North Carolina focus on hydrology and vegetation, while habitat for aquatic fauna is not specifically targeted. If wildlife habitat is part of a mitigation site design, it is not monitored after restoration or construction. Stream mitigation providers express concern over losing credit if results are tied to macroinvertebrate or fish success. North Carolina DWR has changed its mitigation guidance to offer an increase in credits to mitigation providers that do macroinvertebrate/fish data collection and water quality monitoring in streams, to encourage data collection on aquatic fauna in restored streams. This does not currently apply to wetlands.

A literature review on aquatic fauna studies in North Carolina mitigation wetlands indicated that few studies on amphibians (Petranka and Holbrook 2006) and none on aquatic macroinvertebrates in North Carolina wetland mitigation sites have been published. Therefore, our knowledge of how effective current mitigation requirements are for aquatic faunal populations is based on speculation. Hydrology success criteria require mitigation sites to maintain groundwater measurements to a minimum of ≤12 inches from the surface for 5% to 12.5% (consecutive days) of the growing season as of 2016. Vegetation success criteria are typically defined strictly in terms of surviving trees per acre (260 stems per acre by year five or 220 stems per acre by year seven). Our study wetlands, including mitigation wetlands, all exceeded a 12.5% hydrology requirement, often by large amounts (Table 32), suggesting that the minimum success criterion may not be sufficient to support healthy macroinvertebrate and amphibian populations.

A hydrology requirement focused on below-ground water levels only during the growing season presents a complication when considering amphibians. Amphibians need above-ground standing water for a certain number of weeks or months to complete their metamorphosis. Though percent hydrology within 12" of surface and percent days with standing water were highly correlated (Pearson's correlation: $p<0.0001$), there can be instances where a wetland satisfies the hydrology criteria without having any standing water during the year. Our study sites with the lowest percent hydrology within 12" of surface (Block O – 23%, Slate Circle – 27%, and Tiger Pond – 16%) had standing water 25%, 32%, and 32% of the time (respectively), and the shortest hydroperiods of 3-5 months.

Inconsistency in the relationship between hydrology (12" below surface) and amphibian richness, site ranking, or AQAI indicates that habitat suitability depends on more than just growing season hydrology. However, hydrology, particularly standing water, remains a foundational factor; without sufficient water, pond-dependent amphibians will not breed, even in the presence of other optimal habitat or landscape characteristics.

The amphibian breeding season often occurs outside of, or overlapping, the vegetation growing season, which makes it difficult to translate percent growing season hydrology into a hydrology sufficient for amphibian breeding. Total hydroperiod length is more important to successful amphibian breeding; our study indicated an optimum length of 7-8 months of the year (Figure 102). Short hydroperiods of 3 or 4 months exclude many amphibian species.

Not all amphibian species were detected all years, even with repeated site visits during the reproductive seasons and with frogloggers installed and recording every evening. This underscores the importance of longer term amphibian studies, as their occurrences are notoriously dynamic (Hecnar and M'Closkey 1996). Monitoring of amphibians, along with vegetation success, is recommended for the full required monitoring period on mitigation sites. [In North Carolina, the NC DOT and private mitigation providers currently monitor mitigation wetlands for 5 years. Mitigation banks and the In Lieu fee program provider monitors mitigation wetlands for 7 years.] Monitoring efforts should also take into consideration the effect of fish on amphibian species, before drawing conclusions based on amphibian surveys.

Incorporating amphibians as part of the required multi-year monitoring period for mitigation sites could yield valuable information as a site matures. It can take at least four years for amphibians to colonize newly restored wetlands (Pechmann et al. 2001, Sacerdote 2009). However, some species can colonize a created wetland within a year of construction, in addition to returning to the filled wetland site (Pechmann et al. 2001). Rowe and Garcia (2014) found a positive association between anurans and restored site age, suggesting that restored wetlands may become more valuable with time. When natural wetlands are preserved as part of an overall mitigation site, they can serve as important sources of aquatic biota for restored or created wetlands. Currently, in North Carolina, the extent of preserved wetlands that can be included in a mitigation site is capped around 20%.

HOW DO RE-ESTABLISHMENT SITES COMPARE?

In comparison to closed canopy reference sites in this study, re-establishment sites supported both macroinvertebrate and amphibian communities that were comparable or higher in terms of abundance, density, taxon richness, and diversity. This may be attributable to the presence of some of the conditions that were found to be important in determining macroinvertebrate and amphibian community structure. For macroinvertebrates, these habitat conditions were the presence of longer hydroperiods, emergent and aquatic vegetation, and sphagnum moss. Sites with better macroinvertebrate metrics also had buffers with wide vertical stratification and included broadleaf trees, which most likely served as good sources of litter for their consumption in the wetlands. For amphibians, favorable habitat conditions existing on re-establishment sites were longer (seasonal) hydroperiods, few large trees (especially pines), and less acidic pH.

In Italy, Sartori et al. (2015) found macroinvertebrate community composition to be similar between created wetlands and natural wetlands when the wetlands were constructed for the purpose of creating new habitat. When wetlands were created for wastewater treatment, macroinvertebrate community composition differed from natural wetlands. Habitat heterogeneity (in vegetation) was important in influencing assemblages.

In our study, dissolved oxygen in particular was important in influencing both macroinvertebrate and amphibian communities. Higher dissolved oxygen was correlated with higher amphibian abundance, higher amphibian quality index values, and higher macroinvertebrate richness and diversity. Factors predicting higher dissolved oxygen are typical of open canopy systems rather than closed [lower % canopy (ANOVA $p=0.005$), higher % aquatics ($p=0.003$), higher filamentous algae ($p=0.038$), less medium shrubs/saplings cover (0.5-5m tall) ($p=0.022$), less small shrubs/saplings cover (<0.5m) ($p=0.036$), less total litter cover ($p=0.008$)]. These findings suggest that open canopy habitat conditions that are conducive to higher dissolved oxygen are also better for macroinvertebrates and amphibians. Mitigation design should incorporate open canopy areas.

Open canopy reference wetlands and enhancement wetlands had higher amphibian species richness, abundance, and quality than both closed canopy reference sites and re-establishment sites. Macroinvertebrate taxon richness and diversity were also greater on enhancement and open canopy reference sites than on closed canopy reference sites. Macroinvertebrate taxon richness and amphibian richness were negatively correlated with canopy cover. This emphasizes the need to protect small non-forested wetlands, and restore fire-suppressed open wetlands, particularly in the Coastal Plain where these wetlands are very important sources of biodiversity.

ENHANCEMENT BY CANOPY REMOVAL AND FIRE

Fire exclusion, particularly in the Sandhills and Coastal Plain ecoregions where ecosystems are fire-adapted, has caused the invasion of hardwood species into historically open marsh wetlands. Increased canopy cover in wetlands reduces herbaceous and emergent species, increases litter decomposition which lowers pH, and potentially changes the hydroperiod through increased evapotranspiration. Growing season fires reduce or eliminate hardwood trees and peat buildup, and allow emergent vegetation to dominate wetlands by opening up the canopy. These resulting habitat conditions are more suitable for amphibians of the Coastal Plain.

The goal of the wetland enhancement efforts in this study was to improve amphibian habitat by returning closed canopy wetlands to their historic open canopy state, using fire and/or manual tree removal. After canopy removal, enhancement sites had amphibian species richness levels comparable to open canopy reference sites, but greater abundance. After canopy removal at the enhancement sites, habitat conditions generally were similar to those in open canopy reference sites, except in terms of aquatic plant cover and dissolved oxygen levels, which were greater in reference wetlands.

Efforts to increase suitability of habitat for amphibians were successful. The results of this study show that enhancement of historically open canopy wetlands by canopy removal and fire should be continued, to restore and provide habitat for Sandhill and Coastal Plain amphibians.

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