



Periphyton Monitoring in Big Cypress National Preserve

Protocol Narrative

Natural Resource Report NPS/SFCN/NRR—2019/1911



**ON THIS PAGE**

Air plants (genus *Tillandsia*) dot the trunks of bald cypress trees (*Taxodium distichum*) located in a cypress dome near the Concho Billie Trail, Big Cypress National Preserve.

Photograph courtesy of South Florida/Caribbean Network, National Park Service.

ON THE COVER

Calcareous periphyton mats in Fire Prairie unit, Big Cypress National Preserve.

Photographs courtesy of South Florida/Caribbean Network, National Park Service

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Natural Resource Report NPS/SFCN/NRR—2019/1911

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Change History

Change History Log. This table shows the changes and updates to this protocol over time. Add new rows as needed. See Appendix G and associated tables to explain in detail the pilot sampling activities that occurred during the creation of this protocol.

Revision Date	Author	Changes Made	Reason for Change	New Version #
December 2008	K. R.T. Whelan	Pilot sampling of 41 sites	(1) determine if both diatom and soft algae metrics were needed or if diatoms alone would suffice, (2) determine sufficient differentiation between impacted and unimpacted sites to use periphyton as a long-term indicator of ecologically impacted sites, and (3) determine if community structure differed significantly between diatom/soft algae assemblages found in cypress domes and those found in immediately adjacent prairie marshes.	0.01
November 2009	K. R.T. Whelan	Pilot sampling of 65 sites	(1) provide better understanding of nutrient gradient, as depicted through diatom communities, across the hydrological basins in NW BICY	0.02
December 2010	K. R.T. Whelan	Pilot sampling of 36 sites	(1) detect any temporal variability in the structure of diatom communities at sites that had been repeatedly sampled	0.03
January 2012	K. R.T. Whelan	Pilot sampling of 40 sites	(1) to detect any significant temporal shifts in diatom community structure at the collection sites, (2) to expand sampling to the east by adding a seventh basin: Kissimmee Billy East (KBE), and (3) to investigate proposed new sites for BICY water-quality collection.	0.04
December 2012	K. R.T. Whelan	Pilot sampling of 46 sites	(1) continue the development of the temporal periphyton signal in previously sampled sites as well as the proposed new water-quality sites	0.05

Change History Log (continued). This table shows the changes and updates to this protocol over time. Add new rows as needed. See Appendix G and associated tables to explain in detail the pilot sampling activities that occurred during the creation of this protocol.

Revision Date	Author	Changes Made	Reason for Change	New Version #
November 2013	K. R.T. Whelan	Pilot sampling of 48 sites	(1) add another basin, Bear Island (between impacted basins OK Slough and East Hinson Marsh and unimpacted basin Kissimmee Billy Strand), to provide a better understanding of a nutrient gradient, and (2) to collect a second set of periphyton samples from the sites and analyze them for mat TP content.	0.06
December 2014	K. R.T. Whelan	Pilot sampling of 56 sites	(1) continue the development of the temporal periphyton signal in previously sampled sites, and (2) collect a second set of samples from the sites to analyze them for mat TP content.	0.07
2017-2019	R. Urgelles et al.	Produced the narrative of the protocol, the appendices, the Standard Operating Procedures.	(1) to produce a peer reviewed protocol to base the long term periphyton monitoring on.	1.00

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Executive Summary

This document describes a protocol for monitoring periphyton communities within the Big Cypress National Preserve (BICY). Periphyton is identified as a vital sign in the South Florida/Caribbean Inventory and Monitoring Network's (SFCN) monitoring plan because of its sensitivity to environmental change. Periphyton is a microbial mat community that forms the base of the aquatic food web in South Florida wetlands such as the marshes in Everglades National Park (EVER) and the Big Cypress National Preserve. It is a vital component of these wetlands, contributing to critical functions such as primary production, nutrient cycling, and soil production and stabilization. Periphyton is sensitive to slight changes in hydropattern (water quantity and duration) and water quality. Changes to these two ecosystem processes can quickly alter periphyton community structure (species composition and relative abundance), which in turn can create cascading effects on higher trophic levels. Thus, periphyton is an early and integrative indicator of local and regional changes to hydrology. Periphyton is being used to monitor the success of Everglades Restoration for the Comprehensive Everglades Restoration Program (CERP).

In Big Cypress National Preserve, upstream development and agricultural runoff are altering the quantity and quality of the water entering the northwest portion of the preserve. There is concern these hydrologic changes are adversely affecting the region's flora and fauna. Higher than normal water-column total phosphorus levels have been consistently recorded from water-quality stations routinely sampled from April 1994 through November 2010 in the preserve's northwest region. Numerous studies in the adjacent Everglades National Park have linked water management and increased nutrient loading with immediate changes to the periphyton community. Prior to our pilot monitoring effort, there was little periphyton research conducted in Big Cypress National Preserve, especially in the prairie and cypress-dome communities with a soft-water (low calcium or magnesium ions) source. The results from our periphyton pilot studies suggested that diatom assemblages and periphyton mat total phosphorous (TP) concentrations show statistically detectable changes relatable to water quality and therefore could be used as efficient response indicators of hydrology and water quality in the preserve.

The South Florida/Caribbean Network is implementing monitoring of periphyton communities in the preserve to assess the impact of hydrologic and water quality changes on the diatom community structure and TP concentrations of the periphyton mat in northwestern Big Cypress National Preserve. The specific monitoring objectives are:

- Identify any basins in the northwest section of Big Cypress National Preserve where periphyton community structure and periphyton TP content are different from an oligotrophic (low-nutrient) and unimpacted community signal.
- Document any temporal and/or spatial changes in the periphyton community structure and periphyton TP content showing progression towards an oligotrophic, unimpacted condition or a eutrophic (high-nutrient), impacted condition.

Due to financial limitations, the geographic scope of this protocol is limited to the northwest section of Big Cypress National Preserve, which had the greatest water quality concerns for resource

management. This area was divided into seven basins, which were designated based upon flow patterns and natural or non-natural barriers. Basins were initially designated as either “impacted,” if previous water-quality data suggested nutrient loading; “unimpacted,” if believed to be in a natural state; or “unknown” if water column total phosphorus was higher than long-term preserve-wide background levels (above 15 parts per billion [ppb]; Miller et al. 2004). “Unimpacted” does not mean zero impact, but less impacted than other basins. These initial designations will be revised based on the results of the first few years of sampling using this protocol. The sampling design is a stratified restricted random design in which potential sampling sites are randomly selected within each basin. They are restricted to those safely accessible by road, trail or helicopter; that contain a range of habitats from deeper broadleaf marsh to shallower graminoid habitat; and are no closer than 1 kilometer (0.62 miles [mi]) from another site in the same basin.

Field sampling involves navigating safely to the site, proceeding on foot toward the deeper broadleaf marsh habitat until periphyton is encountered, collecting grab samples, taking three water depth measurements, taking water quality measurements (pH, DO, conductivity), and taking a series of photographs. A visual estimate of the vegetation composition is determined for a 5-meter (16.4-foot [ft]) radius circle measured from where the grab sample is collected.

Periphyton samples for diatom analysis are “fixed” with 3% formalin and then sent to a lab for identification and counting of the diatoms present. Periphyton samples for total phosphorous measurements are sent to a nearby lab as frozen samples.

When sample results are received from the labs, analyses are conducted using ordination methods with all the data and also using simplified indices of the proportion of oligotrophic, mesotrophic, and eutrophic diatoms present as well as the average total phosphorous content.

Acknowledgments

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We would like to especially thank Big Cypress National Preserve (BICY) and its Resource Management staff who, over the years, have greatly assisted in the field effort supporting this vital sign. Many thanks to the following: Ron Clark for financial support of the helicopter cost associated with this project; Robert Sobczak for his wealth of knowledge of the preserve's hydrology; Michael O'Leary, Bill Evans, Fred Goodwin, and the rest of Big Cypress aviation staff, without whose help we could not efficiently conduct this monitoring; and Dawnmarie Snow-Roth for assisting with park housing that supports our field crews. We also thank Annette Johnson, Billy Snyder, Jim Burch, John Kellam, Steve Schulze, and Paul Murphy of Big Cypress National Preserve for equipment and logistical support. We thank Damon Doumlele of Big Cypress NP for providing us with the necessary paperwork and permits that allow us to conduct this kind of work in the preserve. Last but not least, we would like to thank all the South Florida/Caribbean Network staff who assisted in this project, especially those who helped in the field.

Acronyms

ATV:	All-Terrain Vehicle
BICY:	Big Cypress National Preserve
CERP:	Comprehensive Everglades Restoration Plan
EVER:	Everglades National Park
GPS:	Global Positioning System (device)
IHOG:	Interagency Helicopter Operations Guide
NPS:	National Park Service
OAS:	Office of Aviation Safety
ORV:	Off-Road Vehicle
PPE:	Personal Protective Equipment
QA/QC:	Quality Assurance/Quality Control
SFCN:	South Florida/Caribbean Inventory and Monitoring Network
SOP:	Standard Operating Procedure
NMDS:	Non-metric Multi-Dimensional Scaling
TP:	Total Phosphorus
UTV:	Utility Task Vehicle

Background and Objectives

Measuring the general health, ecological integrity, and restorative capabilities of ecosystems is a major challenge for environmental managers. The National Park Service (NPS) has initiated a long-term ecological monitoring program, known as Vital Signs Monitoring, to determine the status and the trends in selected indicators of the condition of natural resources in national parks and to provide early warning of abnormal conditions that require management attention. The National Park Service defines vital signs as a subset of biotic and abiotic elements and processes of park ecosystems that represent the overall health or status of a national park's resources.

The South Florida/Caribbean Inventory and Monitoring Network (SFCN) identified 44 vital signs for seven national park units in South Florida and the Caribbean. These vital signs were selected through a scoping of ecological and management issues for each of the parks (Patterson et al. 2008).

Periphyton was selected as an indicator of water quality and low trophic level ecological health in the wetlands of Big Cypress National Preserve (BICY) and Everglades National Park (EVER). Because there is an existing monitoring program for periphyton in Everglades National Park as part of the monitoring plan for the Comprehensive Everglades Restoration Plan (CERP), the network has focused their periphyton monitoring efforts in Big Cypress National Preserve, while consulting and collaborating with the approach used in Everglades National Park. This document explains why periphyton microbial communities are an important vital sign indicator, and details the protocol for monitoring periphyton in Big Cypress National Preserve.

The regional periphyton monitoring for the Comprehensive Everglades Restoration Plan that occurs in Everglades National Park and other nearby areas is similar to the general monitoring program that we have designed but there are some distinct differences. The Everglades monitoring program annually samples from September to December over a much larger area. The region includes: Lake Okeechobee, the Arthur R. Marshall Loxahatchee National Wildlife Refuge, Pal Mar, Holey Land Wildlife Management Area, Water Conservation Area 2 and 3, Pennsuco, and in Everglades National Park: Lostman's Creek, Southern Marl Prairie, Shark River Slough, and Taylor Slough (Marazzi et al. 2017). "Using generalized random-tessellation stratification (Stevens and Olsen 2004), we sampled sites drawn from a pool of GPS coordinates (Philippi 2005) in representative locations (800 × 800 meter [2,625 × 2,625 ft] principal sampling units, PSUs)" (Marazzi et al. 2017). In the SFCN periphyton protocol, we use restricted random sampling within basins of interest and our representative locations are tied to broad leaf marshes.

The CERP periphyton sampling occurs as part of a fish-monitoring program and all periphyton are collected from within a 1 cubic meter (35.3 cubic foot [ft³]) throw net. A 120 milliliter subsample is taken from the homogenized material and frozen for preservation. From the subsample, the periphyton community composition is determined by counting at least 500 algal units from a sealed wet mount microscope slide prepared from a 0.1–1.0 milliliter sample (Marazzi et al. 2017). Additionally, a subsample is taken to determine mat TP concentration (µg/g dry weight) by means of colorimetry after dry combustion. We collect our periphyton as a grab sample within a 5 meter (16.4 ft) radius area. The goal of the grab sample collection is to collect approximately 120 milliliters (4.1

ounces [oz]) of periphyton (two 60 milliliters [2 oz] samples; one for periphyton composition and one sample for TP). We composite five grab samples over the 5-meter (16.4-ft) radius sample area to get the 120 milliliters of sample. Periphyton can be present as calcareous floating mats, on the ground, or as filamentous green algae. The preferred order of collection is (1) floating mat, (2) algae on plants (sweaters), (3) algae on sediments (benthic mats), and (4) algae on woody debris. At some sites, there is an abundance of benthic floating mat to choose from, while at other locations, there is minimal periphyton so we collect flocculent detritus from the benthos and have to search a larger area. The determination of the periphyton community composition in the SFCN protocol follows a similar method to the Comprehensive Everglades Restoration Plan; but currently uses a different lab that makes permanent slides and requires the periphyton samples be fixed in formalin. The same lab conducts periphyton mat TP determination for both programs.

Implementation Park: Big Cypress National Preserve

Big Cypress National Preserve, the first national preserve in the National Park System, was established in 1974, with an additional 146,000 acres (59,084 hectares [ha]) added in 1988. Currently, the park has 720,567 acres (291,562 ha) and receives approximately 425,000 visitors annually. Park visitors come to hike, canoe, camp, bird-watch, hunt, fish, and use off-road vehicle (ORV) trails. Extraction of oil, gas, and minerals occurs within the preserve. Big Cypress National Preserve also has a rich cultural history, is home to the Miccosukee Tribe of Indians of Florida and the Seminole Tribe of Florida, and sustains resources important to their culture.

The preserve contains a large remnant of natural wetland mosaic including cypress strands and domes, pine forests, wet prairies, marshes, sloughs, mangrove forests, and hardwood hammocks. These habitats support a diverse array of flora and fauna unique to South Florida's climate, including 91 federal and state-listed plant species and 31 listed animal species such as the Florida panther and red-cockaded woodpecker. The preserve also contains large stands of dwarf cypress, as well as rare orchids, bromeliads, and ferns. The name "Big Cypress" refers to the vast expanse of cypress rather than to the size of the trees. The larger bald cypress trees (*Taxodium distichum*) were logged during the past two centuries. The few remaining giants are extremely old; some hundreds of years old with trunks over six feet (1.8 meters [m]) wide. The primary management concerns today relate to restoration of the human altered regional hydrology to a more natural flow pattern, improving water quality entering the park, managing invasive species, balancing recreational and extractive uses with long-term sustainability of the system, managing effects from urban development outside and in-holdings within the park, and protecting and preserving rare species.

Rationale for Selecting This Resource

Periphyton is a ubiquitous and ecologically important feature of South Florida wetlands, such as those found in Everglades National Park and Big Cypress National Preserve. The periphyton community is composed of algae, bacteria, fungi, floating plants, and microfauna (Browder et al. 1994; Donar et al. 2004). The epiphytic algal matrix that forms the periphyton mat includes many taxa of cyanobacteria (blue-green algae), green algae, and diatoms (Browder et al. 1994). It is the extracellular polymeric substances (composed of polysaccharides, proteins, and lipids) which are secreted by algae and bacteria that give structure to the periphyton mat (Bellinger 2010). This mat

plays a major role in the energy flow and nutrient cycling of wetland ecosystems and is a critical basal component of the food web, where it serves as a primary food source for small fish, crayfish, grass shrimp, and other small consumers (Gleason and Spackman 1974). When found in large quantities, periphyton accounts for a substantial proportion of the primary production in Everglades' marshes (Browder et al. 1982; McCormick et al. 1998; Ewe et al. 2006). Periphyton is also responsible for the creation of calcitic mud (marl), which is precipitated by cyanobacteria (Browder et al. 1994). Finally, periphyton contributes to the oxygenation of the water column (McCormick and Laing 2003), stabilization of the sediments (Thomas et al. 2006), and control of nutrient upwelling through phosphorus uptake (McCormick et al. 2006).

Periphyton is sensitive to environmental conditions and changes compositionally in direct response to the two main wetland ecosystem drivers (stressors): water quantity and water quality (McCormick and O'Dell 1996; Pan et al. 2000; McCormick et al. 2001; McCormick et al. 2002; Gaiser et al. 2006; Gaiser et al. 2013). Periphyton mats have a high affinity to phosphorus (McCormick et al. 2001; Noe et al. 2002), an essential nutrient which limits productivity in Everglades National Park and Big Cypress National Preserve marshes, and has been shown to respond quickly and predictably to above-background concentrations (Gaiser et al. 2006). Slight changes in water-column nutrient levels, for example, can be rapidly reflected (days to weeks) in the periphyton's community structure, biomass, chlorophyll, and enzyme activity (e.g., alkaline and acid phosphatase). This sensitive indication of phosphorus enrichment provides an early detection to wetland eutrophication before other ecological changes are apparent.

"Detecting change quickly in this system is important, as the difficulty and cost of restoration increases with the duration of damage" (Gaiser 2009). Sustained low-level phosphorus enrichment can lead to a rapid demise of the biodiverse calcareous mats (Stevenson et al. 2002; Gaiser et al. 2004; McCormick and O'Dell 1996) characteristic of areas unaffected by phosphorus enrichment. These mats are subsequently replaced by a community dominated by filamentous green algae, which contains relatively few taxa (McCormick et al. 1996). Shifts in periphyton community structure produce cascading effects throughout the associated animal communities (invertebrates and fish) that depend on the algal biomass for food (Gaiser et al. 2005). Understanding these changes is critical to determining causes of alterations in communities of charismatic megafauna (i.e., large fish, wading birds, alligators). Thus, periphyton serves as an early responder, warning resource managers of impending change (Gaiser et al. 2005), as well as an integrative indicator (Gaiser 2009) that is feasible to monitor and is scientifically defensible (Gaiser 2009).

Periphyton algal assemblages (diatoms, soft algae) have been identified as a relevant metric for landscape connectivity, or intactness of the system (Stevenson et al. 2008; Gaiser 2009). Assemblages are used as indices of biotic integrity in wetlands outside of South Florida (Hill et al. 2000) and changes to their community structure have been causally linked to alterations in hydrological factors (McCormick and Stevenson 1998). Changes in water quality, for example, are significantly correlated with diatom species abundance and community composition (Cooper et al. 1999; Gaiser et al. 2006). Although several other metrics (total phosphorus and ash-free dry biomass) provide a reliable measure of periphyton response to hydropattern and water-quality changes in a

system, diatom species composition has long been used to assess changes in water quality because diatom species can be accurately identified and some diatom species are extremely sensitive to environmental conditions (Stevenson et al. 2010). Diatom community composition is much more sensitive to total phosphorus (TP) than diatom biomass (Pan et al. 2000) and studies have shown that at water-column TP concentrations above 10 parts per billion (ppb), the composition abruptly shifts from a community of oligotrophic diatoms to one dominated by eutrophic taxa (McCormick et al. 1996). This type of community assessment allows for an interpretation of anthropogenic disturbances and comparison to other regional monitoring currently underway, such as the Comprehensive Everglades Restoration Plan (CERP).

These links to periphyton are established in a number of locations of varying spatial scale and ecological community types in Everglades National Park. However, there has been little periphyton research conducted in the preserve, especially in the soft-water communities (e.g., prairies and cypress domes) which occur there. Upstream development, agricultural runoff, and possibly pesticides, may be affecting hydrology and water chemistry in the northwest portion of Big Cypress National Preserve through waters entering the preserve (Figure 1).

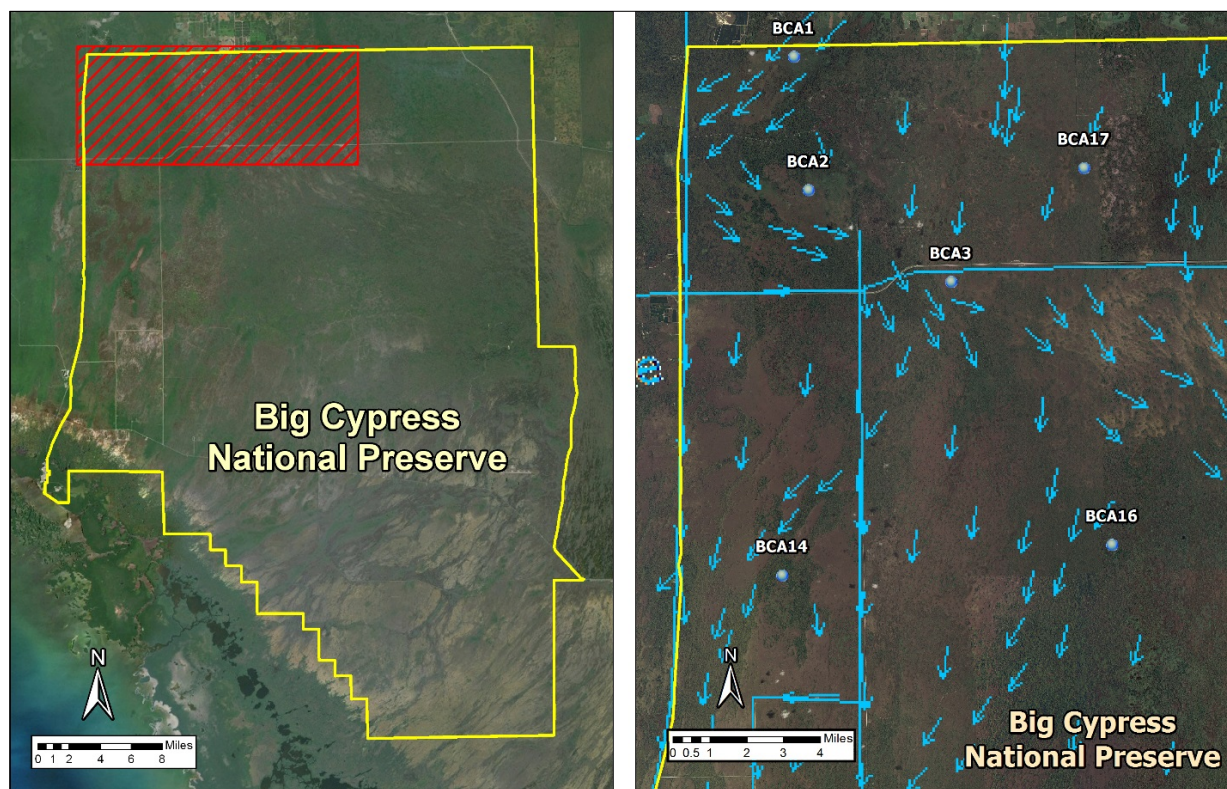


Figure 1. Left, Big Cypress National Preserve with the red shaded area in the Northwest region indicating the area of concern. Right, water flow in northwest Big Cypress National Preserve as represented in Klein et al. (1970).

Although there is no set standard for TP concentrations in Big Cypress National Preserve, natural background levels in the preserve are generally higher than in Everglades National Park, possibly

because of higher TP concentrations already present in natural sources (Miller et al. 2004). Water-quality measurements taken in the preserve from 1991 to 2000 show most median values of TP were slightly greater than 15 parts per billion (Miller et al. 2004). However, from 2001 to 2010, water-quality stations in northwest Big Cypress National Preserve, north of I-75 (Alligator Alley), have recorded high TP, at times exceeding 200 parts per billion, from water-column grab samples (Figure 2). Phosphorus concentrations of this magnitude in the water column typically cause drastic changes to flora and fauna (Newman et al. 1996; McCormick and O'Dell 1996). Waters in the preserve are currently designated by the State of Florida as Outstanding Florida Waters and therefore must be monitored and protected (Miller et al. 2004). Thus, there is concern from resource managers regarding these high-nutrient concentrations in the waters of the northwest Big Cypress National Preserve and the effect on the preserve's aquatic ecosystem. The network has responded to this concern through the implementation of long-term monitoring of periphyton focusing on the community structure of diatoms, a major component of the periphyton algal matrix.

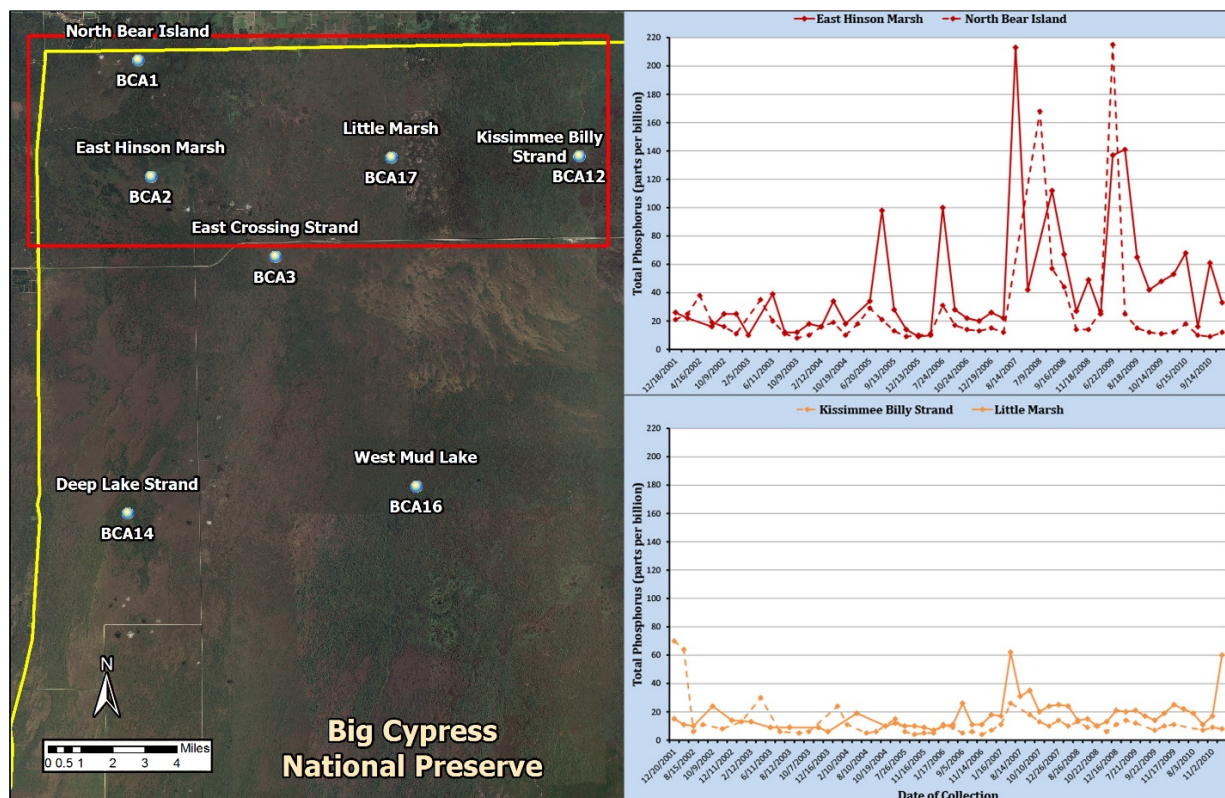


Figure 2. Left, water-quality stations within the area of concern as indicated with a red border. Right, total phosphorus levels over a 10-year period, in parts-per-billion, for the four water-quality stations.

In contrast to water-quality sampling which represents an instantaneous snapshot in time, periphyton community composition is integrative of a time-period previous to the sampling event. Periphyton collection occurs at the end of the wet season. The sampling of the periphyton is timed to capture the effect from the peak overland surface water flow into the preserve. This in-flow of water and

associated nutrients from outside sources occurs at the end of the wet season during peak high water (Robert Sobczak, hydrologist Big Cypress National Preserve, personal communication, 2007).

The time period the active or live diatom community in the periphyton sample collection represents has not been well studied and is not easily determined. The time period depends on the substrate turnover rate and other factors, such as the stability of nutrient inputs over time and the presence and numbers of species at a site. We provide some studies with relevant information below to provide an estimate. Theoretically, the floating mat collection should represent the immediately preceding growing season, containing both the current live diatoms and the early wet season diatoms that have died but whose frustules are still encompassed in the floating mat. When comparing periphyton communities collected from periphytometers (bare collection plates made of Plexiglas® which are collected serially) and nearby floating mat grab samples, it takes about eight weeks for the two methods to begin to have similar diatom communities (Gaiser et al. 2006). Calcareous periphyton communities disappear quickly once nutrients have been added to the system. McCormick et al. (1996 and 2001) reported five-month-post TP enrichment that the loss of calcareous periphyton mat and conversion to filamentous green algae had occurred. Gaiser et al. (2006) reported loss of all calcareous periphyton mat in a long-term dosing project (even for the lowest dosing levels after four years of dosing). Taken together with the above findings, this protocol assumes that periphyton collections will represent the last wet season at a maximum and, provided nutrient inputs are stable, typically at least the two months prior to the collection.

Periphyton as an indicator of water quality is not well-studied in Big Cypress National Preserve. However, the preserve's proximity to monitoring in Everglades National Park and the Water Conservation Areas; its similar low-nutrient, oligotrophic natural condition; and periphyton's prevalence throughout marsh areas in the preserve all suggest that periphyton will be a useful indicator of water quality at Big Cypress National Preserve, similar to its value for the Comprehensive Everglades Restoration Plan. To confirm periphyton's utility as an indicator of water quality, the South Florida/Caribbean Network conducted several pilot studies.

South Florida/Caribbean Network Pilot Projects

Pilot monitoring began in 2008 to explore differences in soft algae and diatom community structure between geographically explicit marsh "basins" in northwest Big Cypress National Preserve. Basins were designated a priori as either "impacted," if previous water-quality data suggested nutrient loading, or "unimpacted," if believed to be in a natural state. Each basin has a corresponding water-quality station and we compared the period of record for water TP concentration to the Preserve background TP level of 15 parts per billion (Miller et al. 2004). At each basin, periphyton grab samples were collected from graminoid marsh sites, preserved, and sent to a qualified laboratory for processing and taxa identification (referencing the USGS BioData database). Significant differences were found between the two basin designations (impacted and unimpacted) using both soft algae and diatom metrics. Ultimately, we determined that diatom assemblages alone can be used as efficient indicators of changes to hydrological pattern and water chemistry (Appendix A).

Use of Diatom Assemblages to Assess Periphyton Quality in Big Cypress National Preserve

For the 2008 pilot year sampling, site selection was based on accessibility from a road or off-road vehicle (ORV) trail, and on flow characteristics. Travel to sites was accomplished by 4×4 pickup truck and then by foot. Sites at subsequent sampling events were accessed by either all-terrain vehicles (ATVs) or helicopter to get a better spatial representation of the preserve.

Subsequent sampling examined both the spatial and temporal aspects of the diatom assemblages at sites throughout each basin. At least six fixed sites per basin were visited annually from 2009 to 2014 to investigate year-to-year shifts in diatom community structure. Starting in 2013, a second periphyton sample was collected from each site to investigate total phosphorus concentrations in the periphyton mat. Non-parametric ordination analysis indicated that sites with similar diatom communities also have similar mat total phosphorus content.

Initially, sites within basins were chosen based on criteria such as ease of access and co-location with water-quality monitoring stations (Appendix B). In 2015, the sampling design was modified to include a random element in site selection. A stratified method was applied in order to randomly select sites from all suitable habitats, allowing for stronger statistical inference and broader spatial coverage of each basin.

The South Florida/Caribbean Network developed the periphyton monitoring in Big Cypress National Preserve protocol to analyze changes in the diatom community structure and total phosphorus concentration of the Big Cypress National Preserve periphyton mat. The monitoring of this important vital sign will allow for detection of the effects of anthropogenic stressors in the preserve, as well as comparison to other monitoring efforts such as those conducted as part of the regional Comprehensive Everglades Restoration Plan (CERP).

Measurable Objectives

The goal of this monitoring is to assess water quality within the non-cattail dominated marsh habitats in northwest Big Cypress National Preserve using diatom and total phosphorus metrics. The South Florida/Caribbean Network will monitor the status and long-term trends in the community structure of periphyton mat diatoms. Additionally, the network will monitor TP concentrations in the periphyton mat within basins of the preserve for the purpose of monitoring changes in water quality that may affect ecological functioning within the preserve. The following objectives will be addressed:

- Identify any basins in the northwest section of Big Cypress National Preserve where periphyton community structure and periphyton TP content are different from an oligotrophic (low-nutrient) and unimpacted community signal.
- Document any temporal and/or spatial changes in the periphyton community structure and periphyton TP content showing progression towards an oligotrophic, unimpacted condition or a eutrophic (high-nutrient), impacted condition.

Link to Management Decision-Making

This protocol does not identify quantitative thresholds to trigger management actions at this early stage in the program. However, waters coming into the preserve should have low phosphorous content such that the natural oligotrophic condition is maintained in the preserve. This protocol makes the connection between water-quality levels in the preserve and effects on the periphyton mat, which is an important base of the food web within this wetland preserve. High water-quality levels of total phosphorous will produce a detectable departure from a natural oligotrophic state of the periphyton community composition and periphyton mat TP content within the preserve. If this occurs, the preserve can use the monitoring data to work with the State of Florida or relevant land owners to bring the levels of phosphorous entering the preserve down to acceptable concentrations.

Sampling Design

Overview of Sampling Design

The South Florida/Caribbean Network applied the results of several years of pilot studies to the development of a sampling design for the northwest portion of Big Cypress National Preserve. The target population is periphyton within mapped graminoid and broadleaf marshes in northwestern Big Cypress National Preserve. The sampling frame has been restricted to mapped and accessible marshes in the northwest section of Big Cypress National Preserve (includes access by helicopter, ORV, truck, or hiking). Sample units consist of the contiguous graminoid and broadleaf marsh habitat within 250 meters (820 ft) of the selected map grid cell centroid from the Western Big Cypress National Preserve Vegetation Map (Whelan et al. 2019).

The area is divided into basins separated by artificial or natural structures that impede water flow during part or all of the year. Each basin is treated as a separate strata or block. Using a restricted stratified random design, a number of potential sites are selected within desirable habitat. Each potential site is evaluated and those that meet specific criteria are established as permanent sites. The design remains flexible and additional basins can be added if time and resources permit.

Rationale for Selecting this Sampling Design

The South Florida/Caribbean Network made a number of critical decisions in order to implement periphyton monitoring within Big Cypress National Preserve. This included limiting the focus to the northwest corner of the preserve, initially planning an annual revisit schedule to all sites, stratifying by basins, focusing on marshes (rather than cypress domes), focusing on a depressional marsh area as the “site” rather than a sub-meter GPS point for re-sampling, using permanent sites rather than new sites each year, using a restricted random approach (rather than judgement sampling), and focusing on diatoms rather than soft algae in the analysis. Some of these decisions were made after analyzing pilot study data.

Limiting Sampling Frame to Northwest Corner of Preserve and Annual Revisit Schedule

Due to financial constraints, the network focused monitoring efforts on the northwest portion of the preserve (Patterson et al. 2008), identified by park staff as the highest priority area due to concerns over the quality of water entering the preserve. In addition, the vegetation map of western Big Cypress National Preserve is complete whereas the map of the eastern portion of the park is still in progress. The level of detail of the vegetation maps allows their use in identifying potential sampling points.

The network has chosen an initial revisit schedule of every year to allow rapid development of a baseline in the area of greatest concern. Provided sufficient resources are available, additional areas of the preserve could be added in a two- or three-year rotation once an appropriate baseline is established in the northwest corner of the preserve. This approach focuses initial and ongoing effort on the area of highest priority and only expands as time and resources permit.

Stratifying by basins

Geographical regions encompassing distinct hydrological flow were delineated following natural and artificial barriers, and were designated as impacted or unimpacted based on water-quality data collected from nearby hydro-stations (Figure 3). For the purpose of this protocol, we use the term “basin” to describe these regions within the preserve. Water basins are either solely rain-driven or have a canal inflow component to them. Basin delineation is based on hydrological flow information (Klein et al. 1970) and locations of associated water-quality stations, both provided by the Preserve’s staff (Figure 1; Sobczak and Murphy, NPS, personal communication, 2008). The basins are described in detail in Appendix C. Stratifying by basins allows increased sampling in basins experiencing water inflow from outside the preserve (and associated greater variability in the data) and allows additional analyses as each basin can be analyzed separately and without the assumption that trends in all areas of the preserve are similar. Utilizing basins also provides an easy and familiar way of reporting results to management. However, using basins results in different selection probabilities of the sample units within each basin (Table 1). Thus the basin grouping must be retained in analyses that analyze change across the preserve.

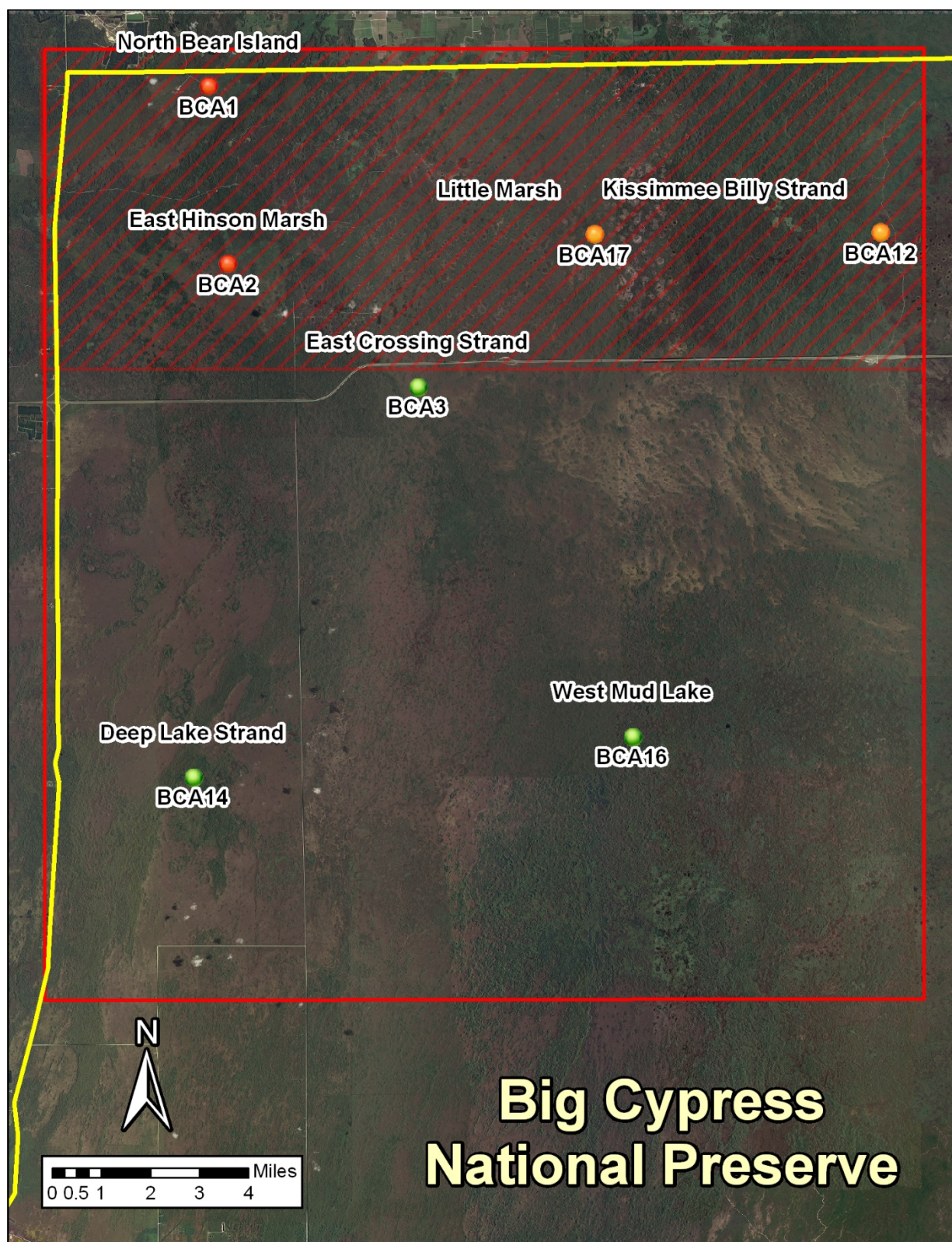


Figure 3. Location of water-quality sampling stations monitored by the Big Cypress National Preserve water-quality sampling program. Area hatched in red indicates area of concern.

Table 1. Number of sites recommended per basin, number of sites rejected and accepted upon evaluation in office and field, and percent of sites accepted. Initial Presumed Status is based on past water quality data from Big Cypress National Preserve (Miller et al. 2004). The mapped sample frame is the mapped area of graminoid and broadleaf marsh and the final column represents the number of sites accepted from the initial sample draw after exclusions occur both in the office and in the field primarily due to accessibility or map inaccuracy.

Basin	Initial Presumed Status	Recommended Number of Sample Sites	Basin Area (hectares)	Mapped Potential Habitat (hectares) ^a	Number Of Sites Excluded In Office Evaluation	Number Of Sites Excluded In Field Evaluation	Final Accepted Number of Sites in Pilot Study	Percent Of Sites Accepted ^b
Okaloacoochee slough (OK)	Impacted	7	3,509	385	6	6	7	37%
East Hinson Marsh (EH)	Impacted	7	5,246	1,435	9	1	7	41%
Bear Island (BI)	Undetermined	6	5,084	781	7	3	6	38%
Little Marsh (LM)	Undetermined	6	5,280	1,222	10	2	6	33%
East Crossing (EC)	Unimpacted	6	5,111	50	21	2	5	18%
Monument (MN)	Unimpacted	6	14,028	1,386	20	0	6 ^c	26%
Fire Prairie (FP)	Unimpacted	6	14,315	604	19	0	6 ^c	27%
Totals	–	44	–	–	–	–	45	–

^a Mapped potential habitat is an estimate of the target population. This area may include some areas misclassified as one of the target wetland types.

^b Percent of sites accepted is an estimate of the percentage of the target population that is within the sample frame.

^c Six plus one alternate.

Limiting Sampling to Freshwater Prairies and Marshes versus including Cypress Domes

In the 2008 pilot year investigation, samples were taken from cypress domes and neighboring prairies at each site to determine if periphyton assemblages responded similarly to nutrient concentrations in both habitat types. In general, no difference was found in results (Appendix B: *Justification for habitat and site selection for periphyton collection in the Big Cypress National Preserve*). However, finding periphyton in cypress domes takes more time due to difficulty of access and diminished physical presence of periphyton in domes compared to prairies and marshes. As the pilot study showed little added value from sampling in cypress domes, this protocol is restricted to freshwater prairies and marshes.

Small Changes of Location around a Depressional Feature have little effect on Diatom Community Structure

This protocol considers areas of contiguous wetland within 250 meters (820 ft) of the centroid to represent the same site. Points should also be within the same depressional feature visited in previous years where logistically feasible. Depressional features are common topographical features in Big Cypress National Preserve (Watts et al. 2014) that are readily apparent at a 1:10,000 scale and typically 100 meters to 1,000 meters (328 ft to 3,280 ft) across (See Figure 4a and b). These are areas of lower elevation with longer hydroperiods than the surrounding habitat and typically have broad leaf vegetation in the deepest areas. Analysis of pilot periphyton data taken across years but with movement of the specific sampling locations up to 320 meters (1,050 ft), found no difference in diatom community structure based on distance (Appendix B). This is consistent with the long-term periphyton data-collection experience in Everglades National Park and the Water Conservation Areas, where communities were found to be more similar across years within the same 500-meter (1,640-ft) sampling unit than across sampling units in the same year (Dr. Evelyn Gaiser, Florida International University, personal communication, 2017).

Allowing a large site provides additional logistical flexibility. The helicopter can land on different sides of the depressional feature, depending on wind direction and speed, and sampling is still within the same sampling unit. In order to minimize the effect of spatial correlation, a restricted random sampling design was selected in which sampling points are restricted to be at least 1,000 meters (3,280 ft) apart in the smaller and more densely sampled basins (Okaloacoochee Slough, East Hinson Marsh, Bear Island, Little Marsh, East Crossing Strand). This distance is expanded to 3,000 meters (9,843 ft) in larger basins (Monument, Fire Prairie). This forces a greater geographic distribution of sampling sites across the basins and helps sampling sites meet the assumption of independence.

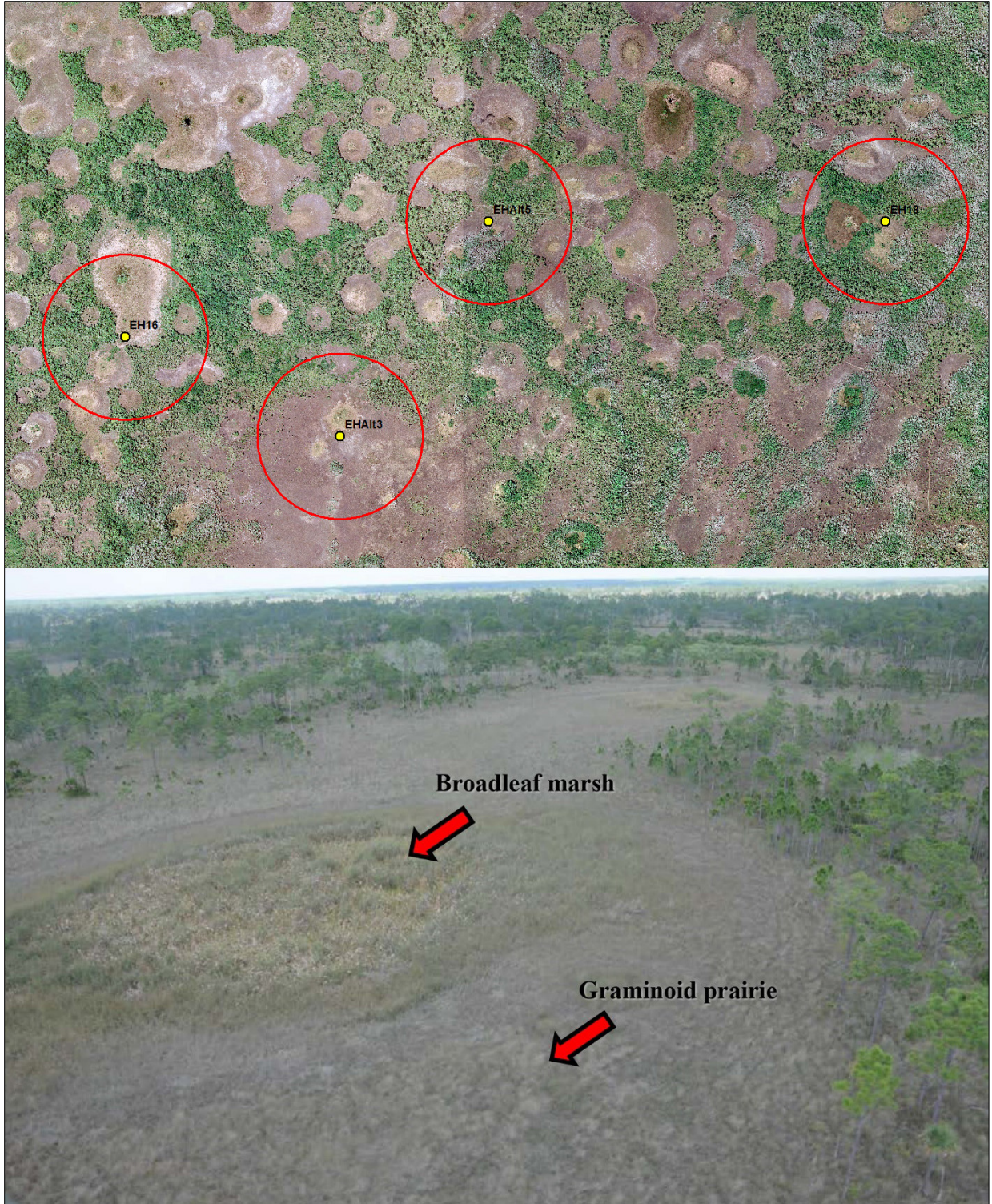


Figure 4a and b. Top (4a): A 1:10,000 scale aerial imagery of Big Cypress showing depressional features represented as circular pock-mark features within the landscape. Red circles are 250 m buffers around randomly selected sample locations (yellow points). Bottom (4b): An oblique aerial view of a typical periphyton sampling location in Big Cypress National Preserve, showing broadleaf marsh surrounded by graminoid prairie.

Permanent Randomly Selected Sites versus Haphazard or Judgement Sampling

During the later pilot studies, sites were selected using a combination of haphazard and judgement sampling. Broadleaf marsh sites were identified from the Big Cypress National Preserve vegetation map or from aerial imagery. The following goals were applied: (a) maximize spatial spread within a basin, (b) co-locate with water-quality monitoring stations where available, (c) answer specific monitoring questions (see Appendix G), and (d) ensure half the sites were accessible via helicopter and the other half of the sites accessible via ORV trails.

After the initial pilot sampling years, early feedback on the sampling design raised concern that sites should be randomly selected to be statistically robust and defensible. Thus, the sampling design was modified to a restricted stratified random design using permanent sites selected within desirable habitat. These sites were assessed for specific criteria such as safe helicopter access at the site. The protocol utilizes permanent sites instead of sites that are re-randomized each year because analysis of the pilot data using paired versus unpaired t-tests suggested that two to seven times as many re-randomized sites would be needed compared with permanent sites to detect the same level of change, depending upon the metric of interest (Table 2).

Table 2. Estimated number of permanent sites versus annually re-randomized sites needed to detect changes (increase or decrease) in total phosphorous ranging from 100-500 ug/g and changes of 50% and 80% of the mean assuming a significance level of 5% and a power of 80% [PS—permanent sites; RS—re-randomized sites].

Basin	# PS to detect relative change from TP mean of 50%	# PS to detect relative change from TP mean of 80%	# PS to detect TP change of 100 (ug/g)	# PS to detect TP change of 200 (ug/g)	# PS to detect TP change of 300 (ug/g)	# PS to detect TP change of 400 (ug/g)	# PS to detect TP change of 500 (ug/g)	# RS to detect relative change from TP mean of 50%	# RS to detect relative change from TP mean of 80%	# RS to detect TP change of 100 (ug/g)	# RS to Detect TP change of 200 (ug/g)	# RS to detect TP change of 300 (ug/g)	# RS to detect TP change of 400 (ug/g)	# RS to detect TP change of 500 (ug/g)
East Crossing (EC)	4	3	3	3	2	2	2	22	10	16	5	3	3	3
Little Marsh (LM)	6	4	5	3	3	3	3	36	15	32	9	5	4	3
Fire Prairie (FP)	6	4	7	4	3	3	3	28	12	31	9	5	4	3
Monument (MN)	7	5	10	5	4	3	3	79	32	128	33	15	9	7
Bear Island (BI)	4	3	6	4	3	3	3	23	10	81	21	10	6	5
East Hinson Marsh (EH)	6	4	51	15	8	6	5	33	14	474	119	54	31	20
Okaloacoochee slough (OK)	7	4	162	43	20	13	9	41	17	1424	357	159	90	58
Average of Basins	6	4	35	11	6	5	4	37	16	312	79	36	21	14

Selection of Periphyton Mat Total Phosphorous and Diatom Community Structure as Key Indicators

The protocol is focused on measuring changes in periphyton mat TP and in diatom community structure. Utilizing TP concentrations in the periphyton mat as a measure is consistent with monitoring conducted under the Comprehensive Everglades Restoration Plan, which uses periphyton mat TP as one of its primary indicators. Total phosphorus also provides the causal link between changes in water TP levels and changes occurring in the diatom community structure. Initially, both diatom and soft-algae characterizations of assemblages were deemed important measures. However, based on the 2008 pilot year results, both components of the periphyton community demonstrated similar results when comparing basins. We determined that an assessment of general ecological conditions of wetlands in Big Cypress National Preserve based solely on diatoms provides a similar amount of information as an assessment with diatoms and non-diatom (soft) algae (Appendix B). The cost savings of only processing samples for diatoms allows for monitoring of more sites for the same analytical costs. Additionally, diatom metrics are more thoroughly developed in the literature than metrics using non-diatom algae, and identification of diatom species is much more precise (Stevenson et al. 2010). Subsequent sampling focused solely on diatom assemblages.

Sampling Frame

The target population is periphyton within mapped graminoid and broadleaf marshes in northwestern Big Cypress National Preserve. The sampling frame has been restricted to mapped and accessible marshes in the northwest section of Big Cypress National Preserve (includes access by helicopter, ORV, truck, or hiking). If additional funding becomes available, sampling can be expanded into other areas of the preserve after the remaining portions of the Big Cypress National Preserve vegetation map are complete. The current spatial extent of sampling is limited to seven basins (Figure 5) that are largely consistent with pilot work conducted to develop this protocol. These basins are:

- Okaloacoochee Slough (OK)
- East Hinson Marsh (EH)
- Bear Island (BI)
- Little Marsh (LM)
- East Crossing Strand (EC)
- Monument (MN)
- Fire Prairie (FP)

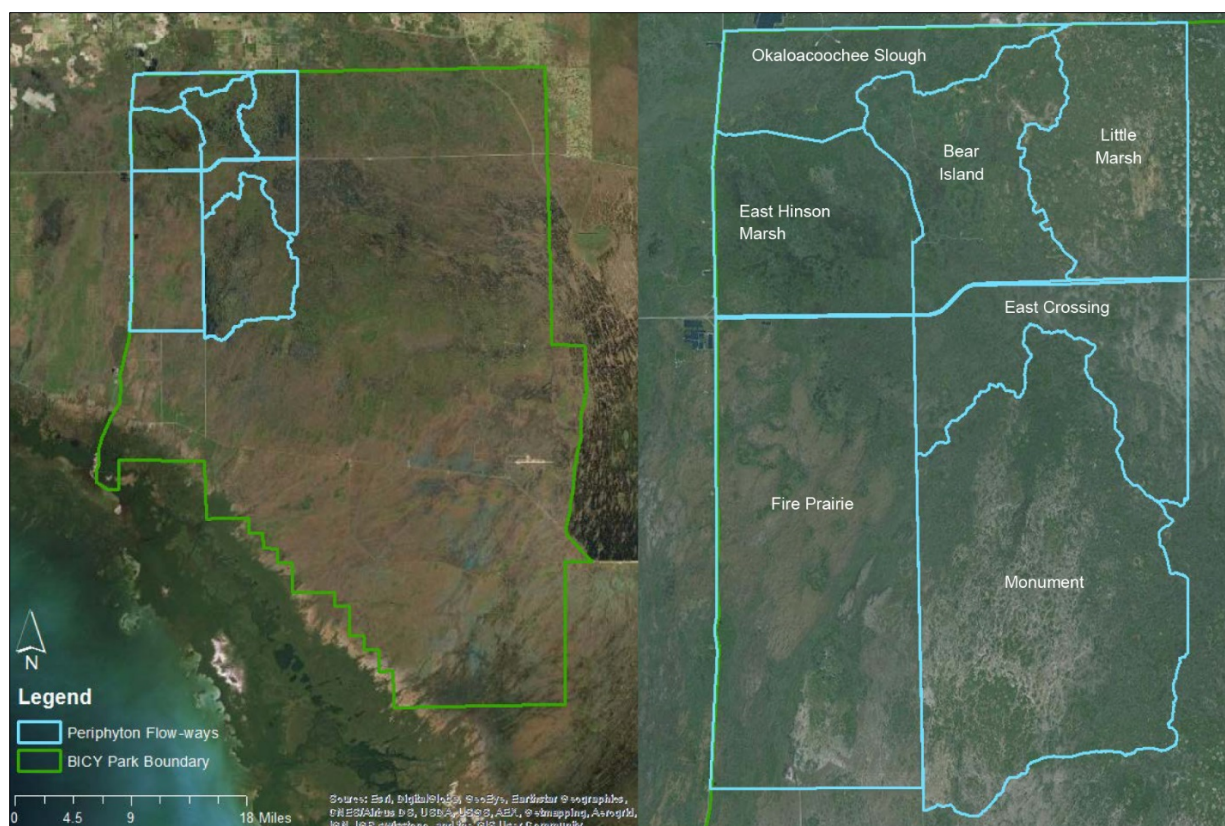


Figure 5. Map showing the seven basins of the periphyton sampling design in relation to the entire preserve (left) and as a close up (right).

These seven basins differ from those used in the pilot study in several ways:

- Areas not covered by the western Big Cypress National Preserve vegetation map are not included in the sampling frame. This includes the easternmost edges of Little Marsh (formerly called Kissimmee Billy Strand), East Crossing Strand, and Monument.
- The Monument basin was extended northward to cover an area in the middle of the sampling frame not previously covered in the pilot study.
- The area formerly called Kissimmee Billy East was part of the pilot work for two years, but is excluded here because it is not part of the western Big Cypress National Preserve map.
- Fire Prairie was extended south to a road (Wagonwheel Road) which bisects the area.

A similar process can be applied to expanding the sampling to the rest of the preserve if additional funding becomes available. However, the eastern 60% of the Big Cypress National Preserve vegetation map is not scheduled for completion until 2019. This map will use a consistent methodology and vegetation classification as the western Big Cypress National Preserve map and unless there is an urgent need, waiting for this map to be completed will be of great assistance, as it will allow a consistent sampling design across the preserve.

Site Selection

The overarching design is a stratified random sample of permanent graminoid and broadleaf marsh sites. Each designated basin is treated as a stratum. Broadleaf marsh habitats in Big Cypress National Preserve appear as circular depression areas (Figure 5), usually surrounded by wet graminoid marsh or prairie, and are defined by dominance of aquatic plant species with flag-like leaves, principally alligator flag (*Thalia geniculata*), duck potato arrowhead (*Sagittaria lancifolia*), and pickerelweed (*Pontederia cordata*) (Davis 1943). Broadleaf marsh habitats usually retain water for most of the year (i.e., hydroperiod greater than 200 days), which allows for sampling to occur early in the dry season as sites become accessible with the recession of water levels.

The South Florida/Caribbean Network used the Western Big Cypress National Preserve Vegetation Map (Whelan et al. 2019) and up-to-date aerial imagery to select sites. This is the most recent and most accurate vegetation map available of the target population. Within each basin, 50 × 50 meter (164 × 164 ft) cells were randomly selected from those with a vegetation attribute of:

- MFG—Graminoid Freshwater Marsh,
- MFB—Broadleaf Freshwater Marsh,
- MFO—Open Freshwater Marsh,
- or various specific types of the above marshes (e.g., sawgrass marsh).

The cells are used to locate areas of interest that typically have a range of habitats from freshwater broadleaf marsh to freshwater prairie (Figure 5). This area of interest is larger than a 50 × 50 meter (164 × 164 ft) cell. Sample units consist of the contiguous graminoid and broadleaf marsh habitat within 250 meters (820 ft) of the selected map grid cell centroid. The overall map accuracy for the Western Big Cypress National Preserve Vegetation Map (Whelan et al. 2019) was 85%. Map accuracy for these specific marsh community types combined was 82% with over 197 accuracy assessment locations. Map accuracy for the specific individual marsh communities was 83%, 75%, 100%, respectively.

Sites were selected in GIS and then evaluated using aerial photography to determine if they meet the following additional criteria. Details of the selection and evaluation process are covered in SOP 16 *Site Selection in ArcGIS—Version 1.0* (Londoño 2019).

- Freshwater broadleaf marsh habitat must be present within a 250-meter (820-ft) radius of the initial randomly chosen point. These broadleaf marsh areas provide a consistent target habitat across the landscape, improving comparability in different basins. While periphyton can technically be harvested from completely dry sites, floating periphyton is preferred to provide a consistent (less variable) sample. In addition, having a range of graminoid habitats means the window of possible sampling will be longer for aquatic invertebrate sampling (which may be added at a later time) as the water gradually recedes from the freshwater prairies back into the deeper marshes.
- The centroid of the randomly selected 50 × 50 meter (164 × 164 ft) vegetation map cell (random selection point) must be 1,000 meters (0.62 mi) or more from other sites within the

same basin (distance is increased to 3 kilometers [1.8 mi] in Fire Prairie and Monument as these areas are more than double the size of the other basins). This will ensure sites are independent and also encourage a greater spatial distribution of sites.

- The random selection point must be greater than 100 meters (328 ft) from a road or trail.
- The random selection point must be within 250 meters (820 ft) of a location safely accessible by helicopter (meeting IHOG safety guidelines of 300 feet [91.4 meter] of open space for the helicopter to land and areas with water depth less than 0.75 meters [2.4 ft; Big Cypress National Preserve Aviation guidance]) or accessible by road (100–500 meters [328–1,640 ft] from road or trail).
- Sites dominated by cattail monoculture (covering more than 90% of wetland area of the 50 × 50 meter [164 × 164 ft] grid cell mapped) will be excluded. While cattails can be present, a dense monoculture shades out the water surface, preventing periphyton growth. Dense cattail stands also have other safety issues as helicopters cannot land in cattail monocultures. This creates an acknowledged bias in the sampling as dense monoculture stands of cattails are known to be indicative of phosphorous enrichment. However, such stands are easily identifiable and mappable via remote sensing. This protocol focuses on areas not already dominated by cattails.
- Sites showing obvious human disturbance within the 250-meter radius, such as used oil pads, are excluded.

Sampling Frequency and Timing

Annual monitoring is conducted approximately two months after the peak of the wet season (typically November or December), when water levels have receded enough to allow helicopters to land and ATVs or UTVs to travel along ORV trails. If certain conditions are met, periphyton diatom communities integrate relevant water-quality conditions for the previous two to three months before collection at a site (Appendix B). In this way, we are following a hydrological clock to determine sampling time based on hydrological stage in which we can sample and to minimize variability associated with hydrological effects on species composition. Monitoring for periphyton mat community composition and periphyton mat TP will be conducted on an annual basis.

Number and Location of Sampling Sites

Sampling consists of six to seven sites sampled per basin at seven basins (44 samples). This number is a compromise between having sufficient replication within the basin and the financial constraints of the long-term monitoring budget (Table 1). Higher sample sizes are recommended in areas that have greater variability or that show effects due to a stressor. Okaloacoochee Slough and East Hinson Marsh showed the greatest variance in results in the pilot studies and are assigned seven sites, while the other basins are assigned six. In the 2018 pilot study, the restricted random sampling design was applied by initially selecting 28–35 random sample locations within each basin and applying the site evaluation criteria in the office and in the field (i.e. presence of habitat visible on imagery, sufficient distance from another accepted point, and accessibility to helicopter, ATV, or hiking). Table 1 shows the results. Accessible points in the East Crossing basin were so rare the team ran out of

randomly selected points and only achieved five acceptable points in that basin. One additional alternate site was also evaluated in each of Monument Basin and Fire Prairie Basin.

Sites are accessed using helicopter, ATVs, UTVs, or hiking. The centroid of the randomly selected cell is used as a starting point. Within a 250 m radius of the starting point, an access point is identified via remote sensing (landing location for helicopter or ATV access). To the extent safely feasible, each sampling event should target the same depressional marsh feature within the 250-meter (820-ft) radius. However, helicopter pilot decisions regarding safe landing locations take precedence and thus the sample unit is considered to be contiguous freshwater graminoid and broadleaf marsh habitat within 250 meters of the selected grid cell centroid. From the access point, the team walks toward the broadleaf marsh area and once the team reaches the graminoid marsh—broadleaf marsh ecotone (which is typically an area with abundant floating mat periphyton), a periphyton sample is collected. The final point must be within 250 meters of the initially selected point and the location of the final sample point is recorded using a handheld GPS unit.

Sampling Level of Detectable Change

Based on analysis of existing pilot data from 2013-2014 using a paired t-test, six or seven sites per basin allows, on average, a detection rate of at least 50% change in periphyton mat tissue TP per basin, a 25% change in relative abundance of oligotrophic diatoms, and a 20% change in relative abundance of eutrophic diatoms, assuming a 5% significance level and an 80% power. Higher sample sizes are recommended in areas likely to have greater variability or showing effects due to a stressor. These minimum detectable differences are smaller than the differences in results between unimpacted and impacted areas in the preserve during the pilot study (Tables 2–4) and will therefore allow the protocol to distinguish the two as well as identify if a basin changes status. The inference of the results is limited to the sampling frame, i.e. periphyton in mapped graminoid and broadleaf marsh in areas that are accessible by helicopter, ORV, or hiking.

Table 3. Values and coefficients of variation (CV; standard deviation / mean) for TP, % oligotrophic diatoms present and % eutrophic diatoms present are provided by basin based upon periphyton data (2013-2014). For total phosphorous (TP) the CV for assuming a permanent site model and a re-randomized site model is provided based on using a paired t-test model or unpaired t-test model.

Basin	# Sites in TP pilot data	TP Mean (ug/g)	TP Permanent Sites CV	TP Re-random Sites CV	% Oligotrophic Diatoms Permanent Sites Mean	% Oligotrophic Diatoms Permanent Sites CV	% Eutrophic Diatoms Permanent Sites Mean	% Eutrophic Diatoms Permanent Sites CV
East Crossing (EC)	8	169	18%	57%	69%	14%	1%	168%
Little Marsh (LM)	7	189	31%	74%	76%	19%	2%	275%
Fire Prairie (FP)	4	211	35%	64%	67%	14%	2%	152%
Monument (MN)	5	255	39%	111%	58%	22%	2%	106%
Bear Island (BI)	5	383	18%	59%	66%	46%	7%	137%
East Hinson Marsh (EH)	5	768	32%	71%	34%	52%	23%	93%
Okaloacoochee slough (OK)	6	1200	38%	79%	40%	32%	17%	89%
Average of Basins	–	453	30%	74%	58%	32%	8%	146%
Range (difference between minimum and maximum)	–	1031	–	–	42%	–	22%	–

Table 4. Estimated number of permanent sites needed to detect absolute changes (increase or decrease) in % oligotrophic diatoms (OD) and % eutrophic diatoms (ED) of 10-30% assuming a significance level of 5% and a power of 80%. # of sites to detect absolute change of XX% (#DAC XX%) for example (#DAC 10%) means # of sites to detect absolute change of 10%).

Basin	OD: # DAC 10%	OD: # DAC 15%	OD: # DAC 20%	OD: # DAC 22%	OD: # DAC 25%	OD: # DAC 30%	ED: # DAC 10%	ED: # DAC 12%	ED: # DAC 15%	ED: # DAC 20%	ED: # DAC 25%	ED: # DAC 30%
East Crossing (EC)	10	6	5	4	4	4	3	2	2	2	2	2
Little Marsh (LM)	19	10	7	6	5	4	4	3	3	3	3	3
Fire Prairie (FP)	10	6	4	4	4	3	4	3	3	3	3	3
Monument (MN)	15	8	6	5	5	4	3	3	3	2	2	2
Bear Island (BI)	73	34	20	17	14	10	9	7	5	4	4	3
East Hinson Marsh (EH)	27	13	9	8	7	5	38	27	18	11	8	7
Okaloacoochee slough (OK)	15	8	6	5	5	4	20	15	10	7	5	5
Average of Basins	24	12	8	7	6	5	12	9	6	5	4	4
East Crossing (EC)	10	6	5	4	4	4	3	2	2	2	2	2

Pilot Study of the Effect of Distance and Comparison with CERP Sample Collection Method

Two assumptions in this protocol are that (a) small distances (less than 250 meters) have minimal impacts on periphyton results, and (b) the grab sample approach used within a 5-meter (16.4-ft) radius is comparable to the 1 cubic meter (35.3 ft³) throw trap method used in the Water Conservation Areas and Everglades National Park as part of the CERP monitoring program. The South Florida/Caribbean Network will test these assumptions in the first year of sampling after publication of the protocol by conducting a pilot study. The pilot study will consist of a transect of samples at a sampling site with samples taken at 0 meters, 50 meters (164 ft), 100 meters (328 ft), 250 meters (820 ft), and 500 meters (1,640 ft). Both the grab sample method within a 5-meter radius circle and the 1 cubic meter throw trap method will be conducted at each distance. One such transect will be conducted in an “unimpacted” basin and the other in an “impacted” basin at sites with sufficient space to conduct the study.

Field Methods

Detailed field methods ensure consistent methodology and repeatability in light of changing personnel (Beard et al. 1999). Aspects of field methodology that are repeated consistently are written in the form of standard operating procedures (SOPs). Standard operating procedures provide detailed instructions to ensure uniformity and consistency in the performance of a given procedure within the protocol. This section describes general methods for gathering data.

As a general overview of methods, sites are accessed by helicopter, off-road vehicle, or truck. Upon arrival, locate where water is present and collect a floating periphyton sample if possible. If the site is dry, then dry periphyton is collected. The sample is placed in a pre-labeled 125-milliliter (4.2-ounce [oz]) Nalgene® sample bottle. The sample is fixed with a 3% buffered formalin solution. Additional site characterization information is collected including vegetation data, water conductivity, and water depth. Pictures are taken at the site. Pictures include one of the datasheet, one of the periphyton mat, and one of each of the cardinal directions when standing at the center of the periphyton collection site.

Big Cypress National Preserve Pre-field Sampling Requirements

The staff of the preserve manage the natural resources within the park and there are a number of legal mandates and directives that must be followed. To meet these requirements we will maintain communication with the preserve resource management chief and any preserve staff whom we are directed to liaison with. All site locations must be reviewed by preserve staff (Ron Clark, NPS, personal communication, 2017). If the site locations are in areas of special management concern (including proposed or designated wilderness), we will apply for administrative permission to sample the site. Since resource management directions may change in the future, the network will annually verify the process with BICY staff at the network's annual meeting. The total phosphorous periphyton mat samples are destroyed during analysis. The periphyton samples collected to determine community composition result in the creation of a permanent slide. These slides will be cataloged and archived according to the preserve's direction.

Field Season Preparations and Equipment Setup

The periphyton sampling in Big Cypress National Preserve has a specific operational season (early dry season—typically November or December). There are three parts to arranging the periphyton sampling season. The first part of the process includes tasks performed prior to any field work. At the beginning of the sampling season, the South Florida/Caribbean Network crew obtains the current stage gage data in the preserve to monitor the hydroperiod dry down. Arrangements are also made for access to sites (some of which are in wilderness areas), housing, and other preparations. The second and third parts include the use of all-terrain vehicles (ATVs) and the helicopter. For helicopter-specific safety requirements, see SOP 2 *Helicopter Procedures for Periphyton Sampling* (Muxo 2019a).

In advance of a helicopter flight, a request is submitted to Big Cypress National Preserve aviation staff and communication is initiated with the resource management staff to make sure the mission is

clear and follows current Big Cypress National Preserve guidelines. Other required flight paperwork is completed and submitted as necessary (SOP 2).

The preparation for ATV use includes ensuring the vehicles are maintained in good working condition and ready for use. At this time, park resource management staff is advised of the mission plan for ATV use within the preserve.

Field equipment includes a handheld GPS unit, personal protective equipment, and field data sheets (SOP 1 *Field Mission Preparation: Creating Datasheets* [Urgelles 2019a]). The South Florida/Caribbean Network is responsible for verifying that participants are equipped with all necessary gear.

Sequence of events during field season

A single monitoring event, from planning to data storage, takes approximately six weeks (Tables 5–6).

- Gather South Florida/Caribbean Network staff. Have pre-field meeting with field technicians. Two to four staff are involved with sampling, generally two for an ATV team and two for a helicopter team. Discuss the sampling plan, individual duties, and project goals. One member of each team will act as project lead to ensure all tasks are completed at each site visited. One member of each team will complete the data sheet while the other person collects samples and takes the necessary measurements at the collection site.
- Make all necessary arrangements with Big Cypress National Preserve and other property owners for access to collection sites.
- Arrive at Big Cypress National Preserve for sample collection. Complete data sheets as samples are collected. Preserve samples for storage after collection.
- Data are entered, checked, and stored while the samples are shipped out for processing.

Table 5. Sampling timeline for helicopter monitoring events.

Personnel	Timetable	Tasks
Project lead	One month prior to flight	• Check Big Cypress National Preserve stage gauges to predict water levels at time of desired sampling • Determine which sites are to be sampled for the year • Contact Big Cypress National Preserve Facilities Management to arrange for park housing; complete the required forms • Review Big Cypress National Preserve flight calendar and contact Big Cypress National Preserve Aviation to confirm flight availability for desired dates • Submit Helicopter Request form and mission requirements to Big Cypress National Preserve Aviation to schedule flights and setup the helicopter
Project lead	One week prior to flight	• Forward to Big Cypress National Preserve Dispatch the flight path along with site coordinates • Check expected weather conditions for flight date(s)
Project lead / team member	One day prior to flight	• Organize required personal protective equipment, camera, GPS, data sheets, sample bottles, and personal locator beacon • Verify operation with Big Cypress National Preserve Dispatch • Fill out South Florida/Caribbean Network office Float Plan • Check expected weather conditions for flight date(s)
Project lead / team member	Day of flight	• Monitor weather conditions • Post South Florida/Caribbean Network office Float Plan • Follow all required safety protocols pre/during/post flight • Ensure pilot and helicopter are certified for mission • Review mission with pilot and flight services personnel • Stow and secure gear
Project lead / team member	Day of flight (upon completion of mission)	• Collect completed flight paperwork from Big Cypress National Preserve Aviation Manager for South Florida/Caribbean Network records • Fix diatom samples with 3% formalin and place TP samples on ice

Table 5 (continued). Sampling timeline for helicopter monitoring events.

Personnel	Timetable	Tasks
Project lead / team member	Post flight (upon return to the office)	• Return all equipment to its proper location • Download cameras and GPS units • Rename and place photographs on Z drive • Scan and store data sheets • Enter data into Access database

Table 6. Sampling timeline for ATV, truck, and hiking monitoring events. This timeline can be used for truck access by substituting truck for ATV.

Personnel	Timetable	Task
Project lead	One month prior to sampling	• Check Big Cypress National Preserve stage gauges to predict water levels at time of desired sampling • Contact Big Cypress National Preserve or Everglades National Park to borrow extra ATVs if necessary • Contact Big Cypress National Preserve Facilities Management to arrange for park housing; complete the required forms
Project lead	Two weeks prior to sampling	• Complete Big Cypress National Preserve ORV Access Form and submit to Big Cypress National Preserve Environmental Specialist for approval
Project lead / team member	One week prior to sampling	• Perform ATV Go/No Go checklist on both ATVs • Ensure ATVs are strapped securely in trailer • Inspect ATV trailer - lights, tires, and connections. • Ensure all tools and accessories are present in the ATV storage boxes • Make sure both ATV spare tires are present in the trailer • Contact Breitburn Energy personnel to notify them of intent to access Big Cypress National Preserve sites through their property if accessing Big Cypress National Preserve via Sunniland area

Table 6 (continued). Sampling timeline for ATV, truck, and hiking monitoring events. This timeline can be used for truck access by substituting truck for ATV.

Personnel	Timetable	Task
Project lead / team member	Daily during sampling events	• Visually inspect ATVs for any damage that may have occurred • Check ATV oil levels and lights regularly • Check trailer tires and lights regularly • Store and secure gear
Project lead / team member	Post sampling	• Thoroughly clean ATVs • Visually inspect ATVs for any damage that may have occurred • Check oil level; and have them serviced if necessary • Check lights • Clean, store, and secure gear • Scan and store data sheets • Download cameras and GPS units • Rename and store photographs on Z drive • Enter data into Access database

Sample Collection

Sites are accessed using helicopter, ATVs or UTVs, or hiking. Appropriate personal protective equipment (PPE) is worn at all times for all modes of transportation. The centroid of the randomly selected cell is used as a starting point. Within a 250-meter (820-ft) radius of the starting point, an access point is identified via remote sensing (landing location for helicopter or ATV access). From the access point the team walks toward the graminoid marsh—broadleaf marsh ecotone (which is typically an area with abundant floating periphyton mat), however, a periphyton sample may be collected anywhere along the way. The final point must be within 250 meters of the initially selected centroid point and the location of the final sample point must be recorded using a GPS unit (Appendix B).

A handheld GPS with all the necessary waypoints and tracks previously uploaded is used to facilitate arriving at the correct destination. On the GPS, a circular radius of 250 meters (820 ft), to match the selected wetland area, buffers each collection waypoint. This circular buffer usually encompasses a heterogeneous habitat and allows for flexibility of collection, since sufficient material may not always be available at the exact waypoint location. It is impractical to spend too much time searching any one site for periphyton; therefore, we have placed a 15-minute time limit to locate the sample within the 250-meter buffer area.

The sampling process consists of grab sample collection, parameter measurements (water depth, conductivity, temperature, and pH), data recording, and photography. A two-person crew (minimum) is required for all site visits, primarily for safety reasons but also to expedite the sampling process. Both team members will carry the required gear needed for sampling. One person will usually complete data sheets (Appendix E; Shamblin 2019a), take photographs and collect information describing the vegetation at the site. The other crewmember will take water parameters, water depth measurements, and collect the grab samples (SOP 4 *Periphyton Sample Collection Methods* [Muxo and Shamblin 2019]). We have found that a two-person team working simultaneously provides an efficient manner of collection.

A periphyton grab sample consists of floating mat, if present. Periphyton mats can be present as floating on the water surface (usually associated with bladderwort) or, if water levels are low, as ground cover. When a floating mat is not readily available, periphyton sweaters can be collected from the stems of submerged aquatic vegetation (SAV). Periphyton mats can also be present in benthic form and collected if neither floating mats nor sweaters are readily available. Benthic periphyton mats are carefully removed from the bottom, including as little surface layer sediment as possible. Some impacted sites may not have calcareous periphyton present and so a sample of filamentous green algae (floating or in sweaters) can be taken in its place. The preferred order of collection is (1) floating mat, (2) algae on plants (sweaters), (3) algae on sediments (benthic mats), and (4) algae on woody debris. The goal of the grab sample collection is to collect approximately 120 milliliters (4.1 oz) of periphyton (two 60 milliliters [2 oz] samples; one for periphyton composition and one sample for TP). Five grab samples from within the 5-meter (16.4-ft) radius sample area are composited to get 120 milliliters of samples. Grab samples are placed inside a 125-milliliter (4.2-oz) plastic Nalgene® opaque bottle (Figure 6) with as little water as possible. Excess water is decanted

prior to fixation. Samples are preserved as quickly as possible upon return from the field (typically at the end of the day or upon return to the helicopter base; no formalin is taken into the field).



Figure 6. Placing a Big Cypress National Preserve periphyton sample into a collection bottle.

Photographs are taken to document the sampling event and can be referred to if there are abnormalities in the diatom data. The photo sequence is as follows:

- Photograph of the data sheet as a place holder;
- Photographs showing site habitat at each cardinal direction;
- Photograph of the water surface or the ground (if no water is present) taken at the collection point, straight down;
- Photograph of the material collected as it goes inside the container;
- Photographs of any unidentified vegetation (if collected, plant should be photographed next to its labeled storage bag); then
- Photographs of any other material of interest.

Data sheets are site-specific (SOP 1 *Field Mission Preparation: Creating Datasheets* [Urgelles 2019a]) and provide aerial imagery with relevant tracks and points of interest, as well as coordinates for the site in case the waypoints are missing from the GPS. Data sheets are printed on all-weather 8.5 × 11 inch in Rite in the Rain® paper. The sample location is determined when entering the area.

Typically, the crew will exit the helicopter in full view of the pilot and move toward the broad leaf marsh area. A sample location is designated at the ecotonal transition zone where there is typically a large amount of floating periphyton. This is the 5-meter (16.4-ft) radius area where a number of grab samples of periphyton are collected and placed in the collection bottle.

At every sample location, a 1-meter (3.2 ft) measuring stick and a pH/DO/conductivity meter (YSI Model 85/YSI Model 63/YSI ProPlus) are used to measure water parameters at the grab sample location. Using the measuring stick, three depth measurements (recorded in centimeters [cm]) are collected (see SOP 4 *Periphyton Sample Collection Methods* [Muxo and Shamblin 2019]) and all three raw values are recorded to represent the grab sample location's average water depth at time of collection. Values of ambient conductivity in microsiemens, temperature in degrees Celsius, and pH are obtained through the use of YSI meters (see SOP 5 *Calibration, Operation, and Maintenance of YSI ProPlus Handheld Water Quality Meter* [Shamblin and Urgelles 2019]) at the grab sample location. Measurements are collected at the center of the grab sample location or the closest area with sufficient water present for the probe to function. All measurements are recorded on the corresponding data sheet.

Habitat characterization is an important component of the data collection. A visual estimate of the vegetation composition is determined for a 5-meter (16.4-ft) radius circle measured from where the grab sample is collected. The crew estimates absolute percent cover of the circle's vegetation, periphyton, and open water (or bare substrate) components. Next, the vegetation component is broken down by the dominant plant species (at least three and up to six), which are also quantified by absolute percent cover.

Post Sample Collection Procedures

Once the samples are collected, they are brought to the SFCN office for sorting, packing, and distribution to the appropriate labs. Currently samples for TP (total phosphorus) analysis are delivered to Florida International University (FIU) and samples for diatom analysis are shipped to Michigan State University (MSU). The South Florida/Caribbean Network retains the ability to use any qualified lab should the need arise to change labs. Samples collected for diatom analysis are preserved in a 3% formalin solution and samples collected for total phosphorus content analysis are placed inside an ice chest. All samples are labeled and include chain of custody paperwork, packing lists, and appropriate shipping labels (SOP 6 *Post Collection Procedures for Periphyton* [Urgelles and Muxo 2019]).

Upon completion of sample processing and receipt of data, payments are made to the corresponding institutions.

Data Handling, Analysis, and Reporting

Data Life Cycle

Periphyton monitoring involves several different types of data that flow through the data life cycle toward a data summary report and certified data products. Figure 7 describes the major steps in the data life cycle and Table 7 provides an overview of the file storage locations and naming conventions. The database was developed in Microsoft Access 2010, and is based upon the Natural Resources Database Template (NRDT).

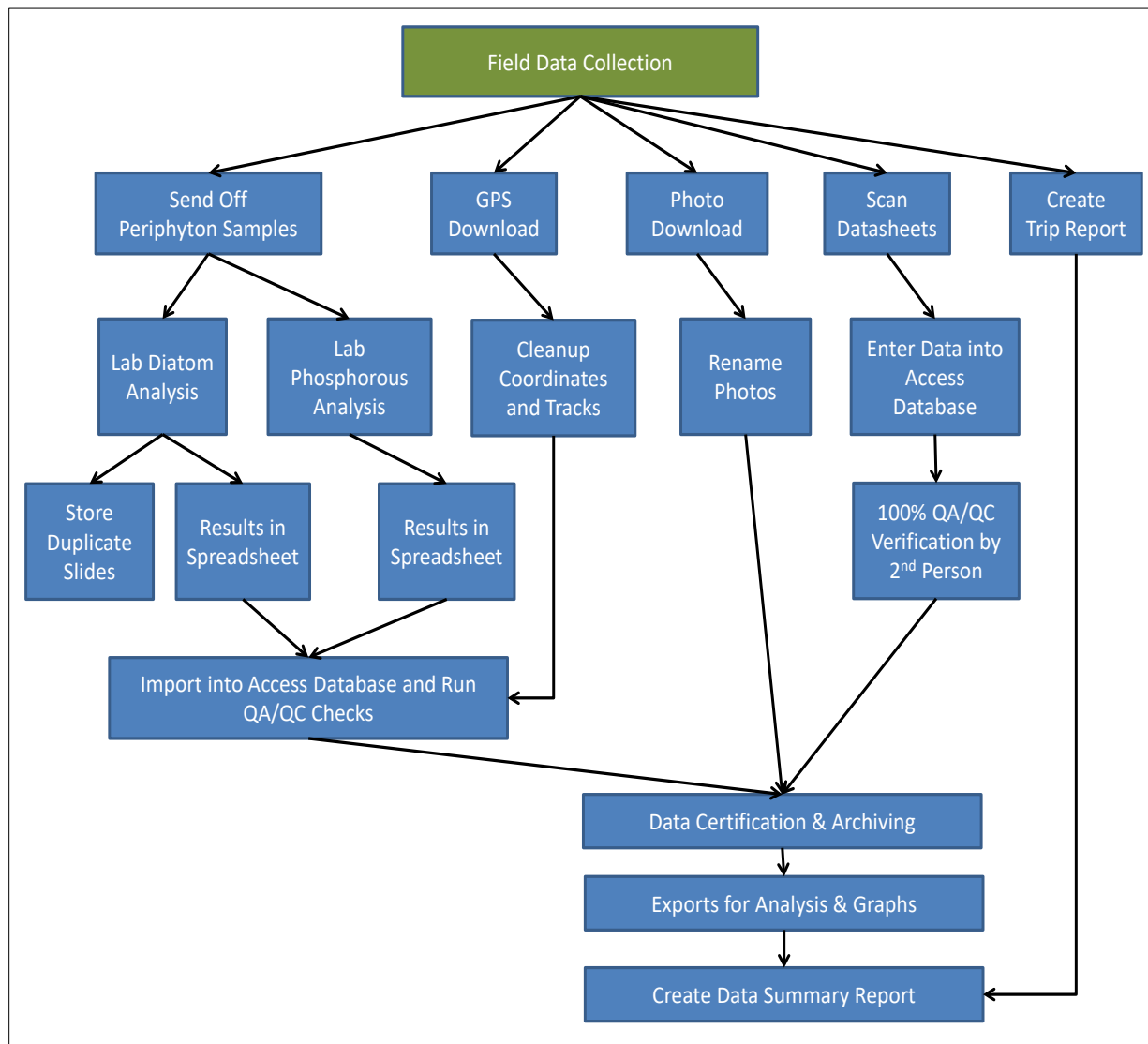


Figure 7. Major steps in the periphyton monitoring data life cycle.

Table 7. Critical field and data management file locations. Instances of “Z:\SFCN\Vital_Signs\Periphyton\” are replaced by “...\”.

File type	Folder	Filename
Flight and safety documents	Z:\Helicopter\Periphyton\ Also in: Z:\SAFETY\ Z:\SAFETY\SFCN_SOPs_with_JH As\JHAs\Community_Group_JHA's \	SFCN_Helicopter_Go_No_Go_Checklist_Mod _USE.docx GAR_Risk_Assessment_Model_Periphyton_U SE.doc Flight_Request_Form_9400_blank_use_this.p df Aviation_Risk_Assessment.docx HAZARDOUS_MATERIALS_MANIFEST.docx Job_Hazard_Analysis_Periphyton.pdf
Blank datasheets	...\documents\blank_datasheets\	YYYYMMDD_periphyton_datasheet.mxd YYYYMMDD_periphyton_site_evaluation.docx
Photos	...\images\YYYY\Site\	YYYYMMDD_BICY_Site_Photonumber.jpg
Scanned datasheets	...\data\scanned_datasheets\YYYY \	YYYY_periphyton_scanned_datasheets.pdf
Diatom lab slides	Stored according to Big Cypress National Preserve policy	–
Lab analysis results	...\data\YYYYMM\Diatoms ...\data\YYYYMM\TP	BICY_YYYY_Diatom_counts.xlsx BICY_YYYY_Periphyton_sampling_TP.xlsx
Database and Tracklines	...\data\ ...\spatial_info\YYYY_location_data\tracks\	SFCN_Periphyton.mdb YYYYMMDD_periphyton_tracks.shp
Statistical Analysis script and results	...\analysis\	YYYYY*.xls or *.primer files
Data Summary Reports	...\documents\summaries_reports\ annual_reports\	YYYYMMDD_YYYY_Annual_report_periphyto n.docx
Protocol	...\documents\protocol\	YYYYMMDD_BICY_Periphyton_v1.docx, *.pdf
Archive	https://irma.nps.gov/DataStore/	Database, scanned datasheets, reports, and analysis code

Data sheets are scanned within a week of each field day, placed in a three-ring binder, and stored in the South Florida/Caribbean Network Conference Room. Scanned copies are placed in *Z:\SFCN\Vital_Signs\Periphyton\data\scanned_datasheets*. Photographs are downloaded, renamed, and stored in *Z:\SFCN\Vital_Signs\Periphyton\images\SurveyYear* (SOP 7: *Photo Downloading and Renaming* [Muxo and Urgelles 2019]). GPS files are downloaded, cleaned, and stored in *Z:\SFCN\Vital_Signs\Periphyton\spatial_info\SurveyYear_location_data* (see SOP 8 *GPS Track and Waypoint Downloading and Archiving* [Shamblin 2019a]).

Database

The periphyton database (Figure 8) is located in:

Z:\SFCN\Vital_Signs\Periphyton\data\SFCN_Periphyton.mdb.

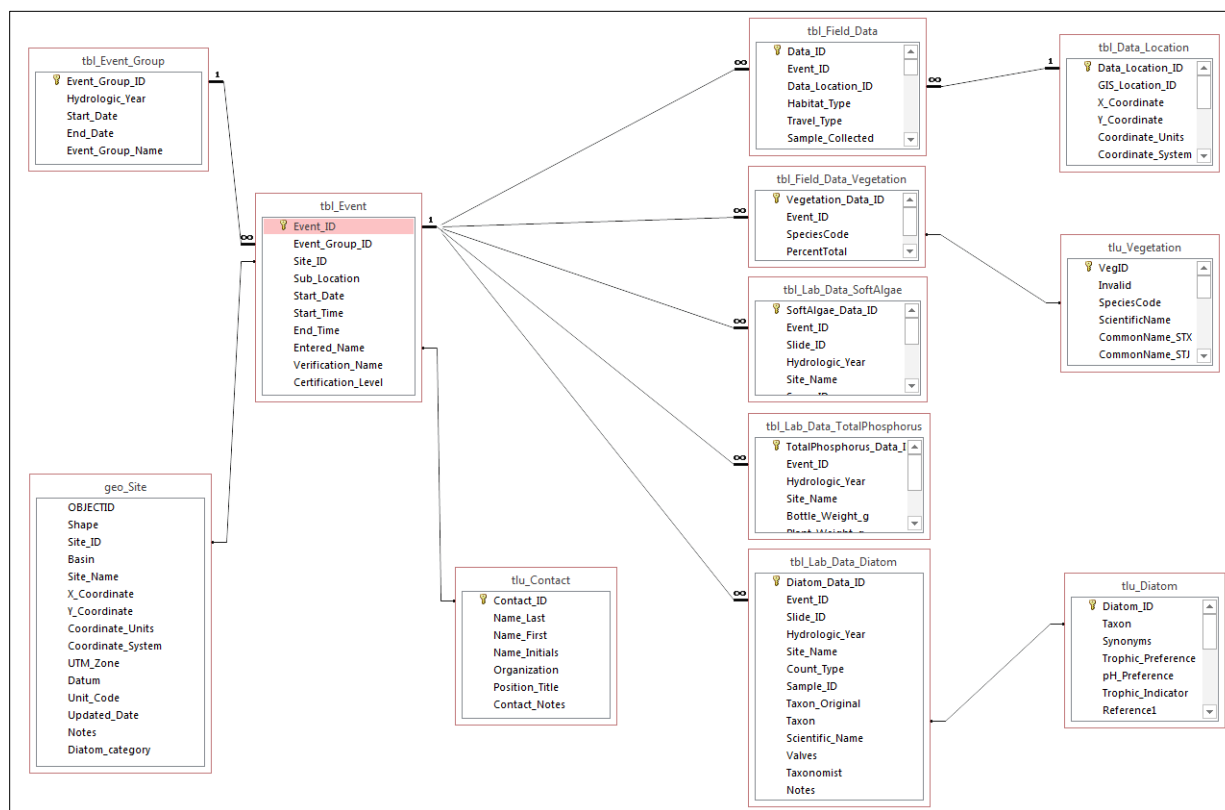


Figure 8. Schema diagram showing the primary tables and relationships within the periphyton database.

The database is a Microsoft Access geodatabase and initially used the Natural Resource Database Template as a guide. The database stores four general types of data:

- Field data—these data are manually entered directly into the Microsoft Access database (SOP 9 *Data Entry and Quality Assurance* [Urgelles and Shamblin 2019]).
- Spatial Data—these geospatial feature classes include Permanent Monitoring Sites, Basins, and Annual Sample Coordinates. The Annual Sample Coordinates shapefile holds new waypoints that are appended after each field season. This process is described in detail in SOP 8 *GPS Track and Waypoint Downloading and Archiving* (Shamblin 2019a). GPS tracklines are stored in shapefiles outside the geodatabase.
- Lab data—these are processed data provided by a contracted lab. They represent the results of any soft algae, diatom, and/or nutrient analysis. These data need to be imported into the database and linked to the Field and Spatial Data (SOP 15 *Importing Laboratory Data* [Londoño and Patterson 2019]).

- Lookup lists—these lists ensure consistency in data entry and reporting. They include information such as taxonomy, project staff, substrate types, and park units.

Individual table and field definitions are provided in Appendix D.

Completed field data sheets are entered into the Microsoft Access Database as soon as possible after returning from the field, while details are still fresh in the mind of the researchers. In general, all field data will be entered into the database no later than one week after the completion of the annual sampling so that an export of the latest data can be attached to the samples when they are shipped for analysis.

The Access database has a data entry form that is designed to resemble the field data sheet for ease in entering the data. The details of data entry procedures for the Access database are provided in SOP 9 *Data Entry and Quality Assurance* (Urgelles and Shamblin 2019).

Quality Assurance / Quality Control

The data quality standards for this protocol are provided in Appendix F.

Taxonomic naming follows the diatom database supported and maintained by [the U.S. Geological Survey, BioData](#). We will document the version used and we will download a copy for reference upon receiving diatom data. To assist with documentation and harmonization, diatom species that make up greater than 5% on the slide are photographed, named, and retained.

Each year five sites will be chosen to serve as quality control (QC) sites for TP. These extra samples will be collected at the same time as the typical field samples, but they will be labeled “Test X” and recorded on the datasheet so only the South Florida/Caribbean Network knows the identity of the sampled sites. The lab will be unaware of which sites these samples come from, thus providing South Florida/Caribbean Network with an opportunity to compare and understand the level of sampling variability at a single site. Five sites will have duplicate periphyton samples collected and sent in for diatom analysis. After several years, these results will be used to estimate sampling variation of the TP and diatom data. After two years of this quality control practice we will assess if the quality control samples can be reduced.

We will minimize lab changes whenever possible. If a change in lab is unavoidable, provide the new lab with the photographic library of diatom species that accompanies the previous sample slides. Provide the new lab with the USGS BioData version used with the last sample for taxonomic consistency. Then provide six of the sample slides from the most recent sampling event to the new lab, including three from eutrophic sites and three from oligotrophic sites. The new lab will process the six slides and the South Florida/Caribbean Network would expect general agreement on ranking of the dominant taxa (taxa making up more than 5% of the sample) and that the sample would have similar percent trophic estimates (percent oligotrophic and percent eutrophic). Large variations in results between the two labs would require further exploration to determine why the discrepancy is occurring.

The database has several built-in features to ensure the integrity of the entered field data. These include a restricted list of plant species codes, which displays a message if the species has not been used before. The opportunity to add new species is still provided, but the pop-up message serves as a safeguard against misspelled entries. Additional lookup lists are used for other fields including sampling sites, substrate location, substrate type, travel type, water color, and sampling personnel.

Once the data are entered into the Microsoft Access database, the person who entered the data reviews 100% of their work to minimize errors of omission or transcription. The next step is to hand the datasheets to a second person for a 100% quality assurance (QA) verification. The verification is done by either printing the data or viewing the data on screen and comparing it to the field sheets. Any errors found are corrected, and frequent or unusual errors are brought to the attention of the data manager and community ecologist to determine if additional checks are necessary. After verification is complete, record the date and name of the person who completed the verification in the database.

There are additional checks to complete on the laboratory data. These include comparing the identified diatoms to the existing diatom lookup table. New species need to be added to the lookup and researched to determine their trophic preference. The summed valve count for the diatom species in a single sample should add up to 600. The TP is checked for concentrations beyond 5,000 $\mu\text{g/g}$. If anomalies in the data exist, this would generate a review by the community ecologist to determine if the data should be marked invalid.

The community ecologist and project lead will examine data outputs and graphs spatially and through time to check for suspicious data (unusual spikes or changes) in an ecological context. Unusual data may trigger checking of ancillary data (pH, conductivity, site characterization photos, field notes) to determine if a potential cause can be elucidated and whether data should be flagged as suspect with a description (e.g., site adjacent to bird rookery).

Metrics

Metrics calculated for this protocol include:

- Periphyton mat total phosphorous content
- Simplified indices of percent oligotrophic diatoms and percent eutrophic diatoms
- Dissimilarity matrix (and related similarity matrix) calculations between all sites based upon abundance of all individual diatom species

Periphyton Mat Total Phosphorous Content

Periphyton mat total phosphorous (TP) content of periphyton samples is analyzed by sending samples to qualified laboratories. Individual site results and basin multi-site averages are used in statistical analyses.

Simplified Indices of Percent Diatoms by Trophic Category

Diatom data are summarized into seven trophic categories (Whitmore 1989; Gaiser 2006) based upon lists provided by Dr. R. Jan Stevenson of Michigan State University and supplemented with additional literature research on local species (Table 8; Figure 9; Appendix F).

Table 8. Diatom indicator categories and definitions.

Indicator category	Definition
Eutrophic	Diatoms only documented in eutrophic conditions
Mesotrophic–Eutrophic	Diatoms documented in the range of mesotrophic to eutrophic conditions
Mesotrophic	Diatoms documented in mesotrophic conditions
Mesotrophic–Oligotrophic	Diatoms documented in the range of mesotrophic to oligotrophic conditions
Oligotrophic	Diatoms only documented in oligotrophic conditions
Oligotrophic–Eutrophic	Diatoms found in all conditions
–	Diatom relationship as indicator of nutrient status unknown

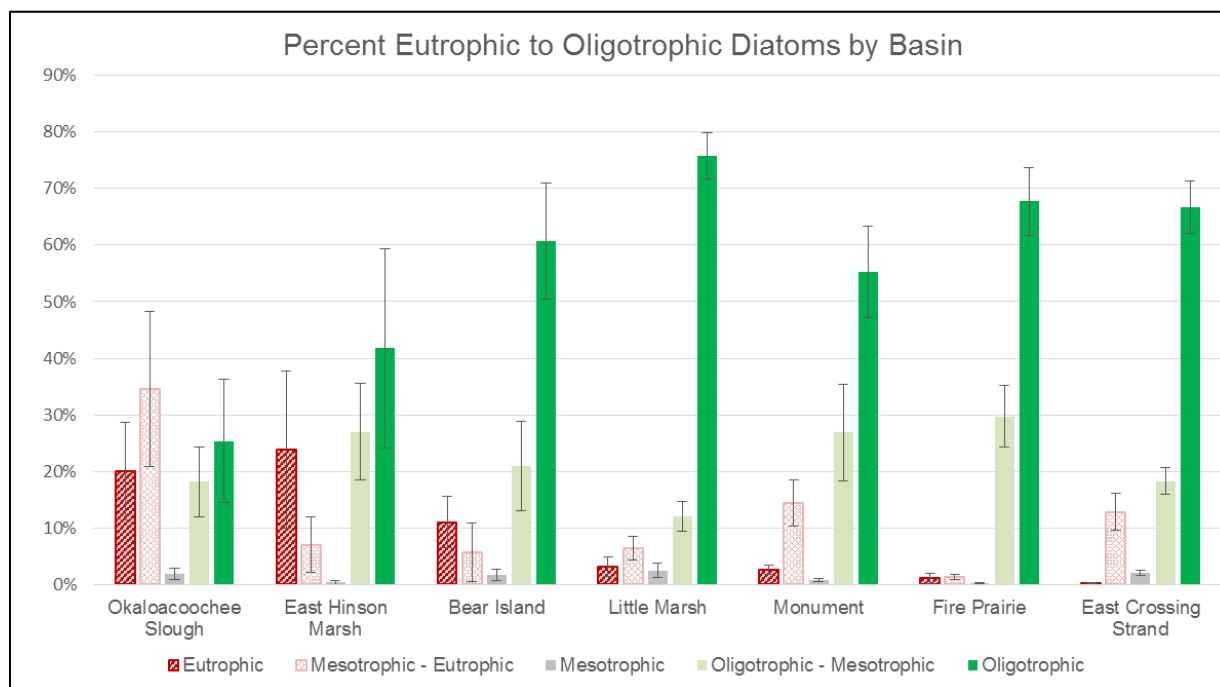


Figure 9. Graphical display of average percent of diatoms classified into five indicator categories by basin: Eutrophic; Mesotrophic-Eutrophic; Mesotrophic; Oligotrophic-Mesotrophic; Oligotrophic. Data represent 2013 averages and standard errors by basin.

Not all trophic category references are from local sources. Table G-1 documents the reference used and is a living document, such that when more localized and relevant references become available, the South Florida/Caribbean Network will update the table. The network will also consult subject matter expert(s) as to which references are more appropriate. Generating this trophic level classification allows analyses to be conducted on indices such as percent eutrophic diatoms and percent oligotrophic diatoms. These indices are calculated as the percent of diatoms with

a given trophic designation divided by the total number of diatoms identified to a trophic designation (i.e., the indices exclude diatoms for which the trophic designation is unknown from calculations).

Dissimilarity and Similarity Matrices Calculated based upon Abundance of All Diatom Species

While simplified indices are easy to understand, they do not take advantage of all the information available in the diatom data and are dependent on the state of scientific knowledge of the trophic categories with which each species is associated. Thus, a dissimilarity matrix (and related similarity matrix) are also calculated based upon abundance of all diatom species to allow multivariate approaches to analyzing the data.

Species-by-sample matrices are generated from the Microsoft Access periphyton database. The matrices consist of species count data (diatoms) for all species for each site per year and are analyzed to identify latent patterns of community structure among the study sites. Matrices are exported into PRIMER (PRIMER-e Ltd., Plymouth, UK), a software package used for community analyses. Diatom data are non-normally distributed because many samples lacked specimens of one or more species, as is common with abundance and count data. The data are standardized to reflect relative densities of species for each site and subsequently applied a square-root or double square-root (4th root) transformation to better represent all species in the samples (Clarke and Warwick 2001).

Whether transformation is needed or not depends on whether the analysis should reflect the rare species or just the common ones. Untransformed data will typically lead to a shallow interpretation in which only the pattern of a few, very common species is represented (Clarke 1993). Thus, applying a transformation sequence (square-root, fourth-root, presence-absence) allows progressively greater contribution from the rarer species (Clarke and Green 1988). Ultimately, the standardized and transformed data are used to generate a sample dissimilarity matrix using a Bray-Curtis dissimilarity coefficient (δ), which produces values ranging from 0 (no dissimilarity) to 100 (total dissimilarity) between each pair of site means (Faith et al. 1987; Clarke and Warwick 2001).

Thresholds

Initial periphyton mat TP thresholds are taken from work in Everglades National Park. Acceptable ranges (green) for TP were set at 200 $\mu\text{g/g}$ or less in Shark River Slough and Taylor Slough (Gaiser 2009). Caution (yellow) was initially identified as 200–400 $\mu\text{g/g}$ in Shark River Slough and as 200–350 $\mu\text{g/g}$ in Taylor Slough (Gaiser 2009). For the purpose of initial reporting, the South Florida/Caribbean Network is using 400 $\mu\text{g/g}$ as the caution (yellow) threshold with the understanding this may evolve to a more Big Cypress National Preserve-specific threshold. Gaiser (2009) and Trexler and Gaiser (2013) suggest using baseline acceptable conditions to determine the cutoffs with one standard deviation being the caution (yellow) threshold and two standard deviations being the altered (red) threshold. As some sites during the pilot study showed values far in excess of 400 $\mu\text{g/g}$, a fourth “very high” threshold will be used in some analyses of greater than 1,000 $\mu\text{g/g}$. Absolute thresholds have not been established for diatom community metrics. As the South Florida/Caribbean Network builds its periphyton collection we will be able to assess the percent oligotrophic species present in a collection to help determine a threshold for a management response following the example advocated by Gaiser et al. (2015) and Marazzi et al. (2017).

Analysis

Classifying Sites and Basins by Nutrient Status

To determine which sites and which basins are different from an oligotrophic (low-nutrient) and unimpacted community signal (objective 1), each site TP and the average TP of each basin will be compared with the published index for the CERP periphyton monitoring program occurring in Everglades National Park (Gaiser et al. 2006; Gaiser 2009) as described previously (see Figures 10 and 11). A fourth level of mat TP greater than 1,000 ug/g may be added as the pilot study showed the presence of sites with values greatly in excess of this level.

As absolute thresholds have not been established for diatom community metrics, SFCN will verify the relationship between periphyton mat total phosphorous level and diatom community structure by using an analysis of similarity (ANOSIM) analysis with TP class as a factor. We can follow this analysis by using a SIMPER analysis to evaluate which diatom species abundances show the greatest differences due to TP level. In addition, the relationship between TP and the percent of eutrophic and percent oligotrophic diatom indices will be verified (e.g., Figure 12).

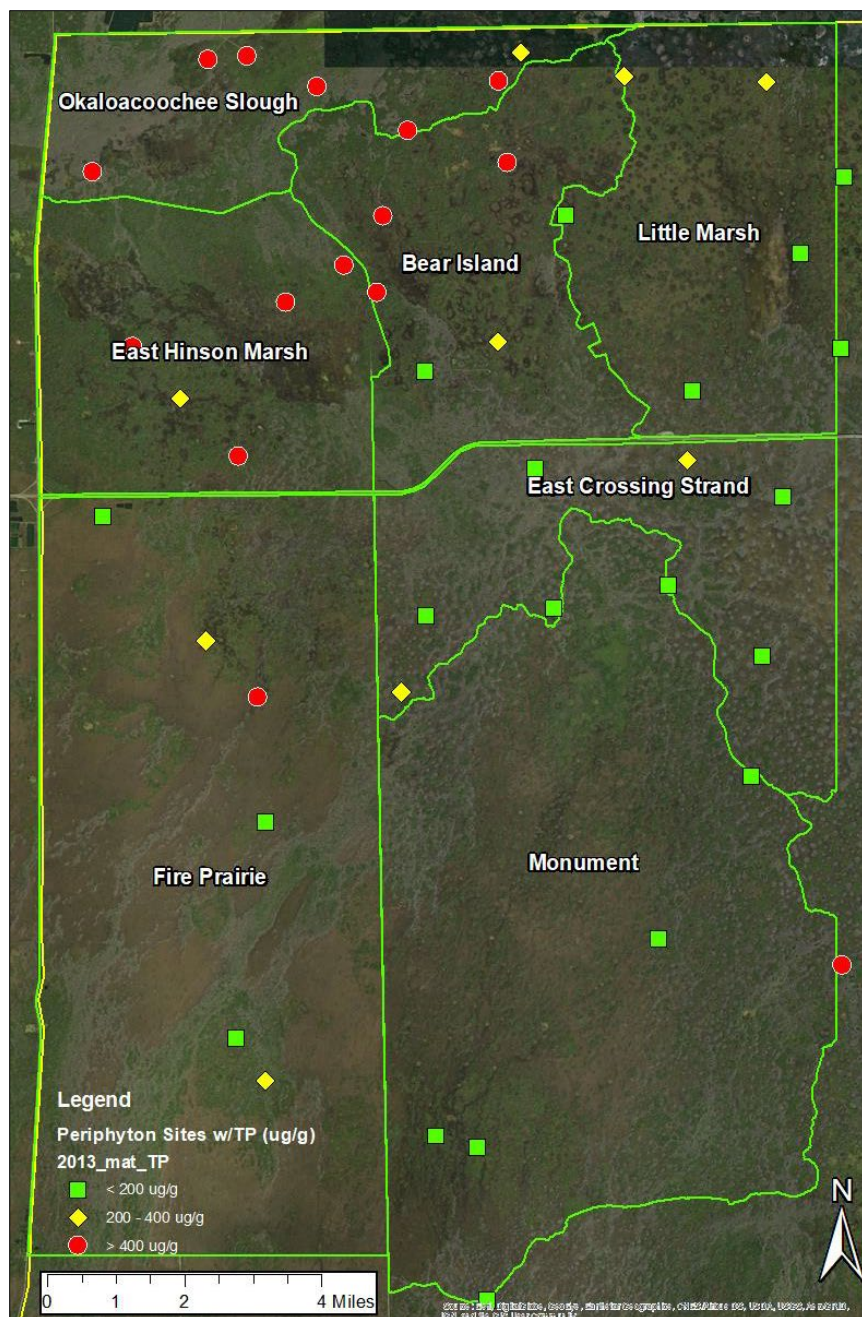


Figure 10. Example of periphyton mat data by site visually displayed on a map. Data represent 2013 values. (For display purposes only).

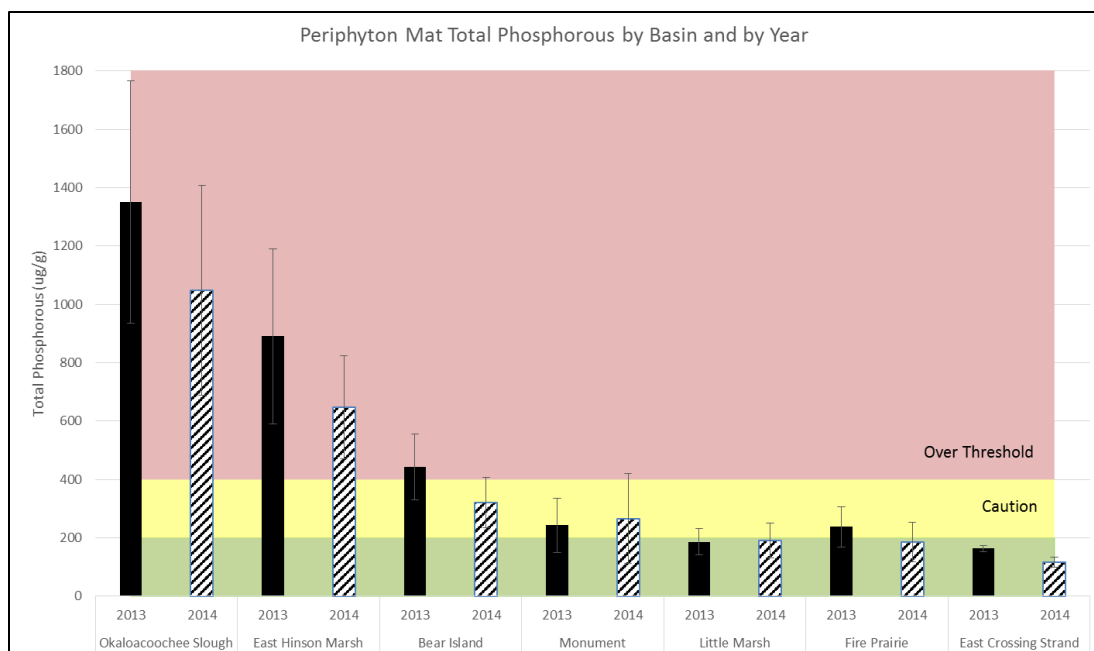


Figure 11. Graphical display of average periphyton mat total phosphorous results by basin and year against background of three thresholds based upon published index for CERP periphyton monitoring program (For display purposes only).

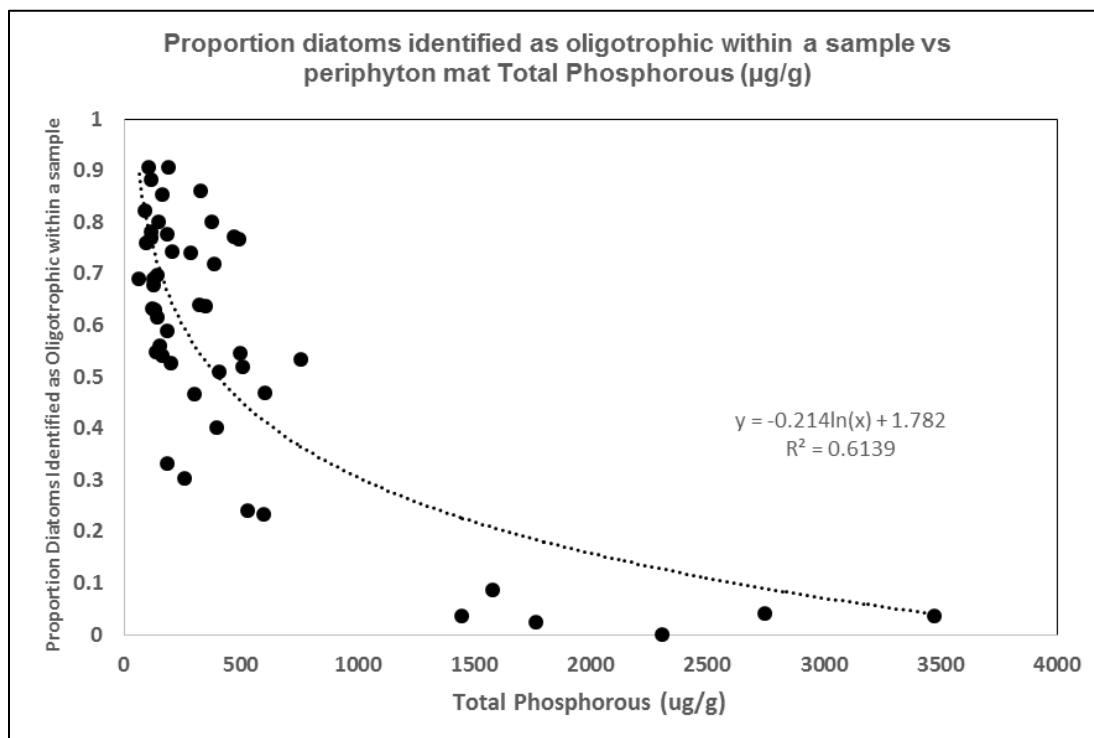


Figure 12. Graph showing relationship between periphyton mat total phosphorous levels and periphyton mat percent oligotrophic diatoms. Data represents 2013 pilot data.

Evaluating Spatial and Temporal Changes in Periphyton TP Content and Simplified Periphyton Community Indices

To investigate the temporal or spatial change in mat TP, percent oligotrophic diatoms, and percent eutrophic diatoms across basins (sites are nested within basin) and across time, a repeated measures analysis of variance or analysis of co-variance will be used coupled with mean separation tests (Table 9). In the likely case of interactions between basins and time, basins may also be analyzed individually using paired t-tests or an analysis of covariance. Results will also be compared graphically (See Figure 9 and 11).

Table 9. Recommended periphyton metrics, associated analyses, and the analysis purpose.

Metrics	Analysis	Analysis Purpose
• Periphyton total phosphorous; • Percent oligotrophic diatoms; • Percent eutrophic diatoms	Parametric tests such as repeated measures ANOVA with mean separation test (Analysis of Variance) or Repeated measures ANCOVA (Analysis of Covariance) or paired t-tests by basin.	Test null hypothesis of no difference among basins; Test null hypothesis of no change due to time; Provide easy to understand indicators for graphs, maps, and reports.
• Dissimilarity Matrix (and related • Similarity Matrix)	ANOSIM (One way or two way Analysis of Similarities)	Test null hypothesis of no difference in diatom community due to mat TP level. Test null hypothesis of no difference within or among basins. Test for change among time steps.
	NMDS (Non-metric Multi-Dimensional Scaling)	Graphical display of similarity and dissimilarity of site communities.
	SIMPER Factor analysis	Groups sites according to factors and determines which species contributes the most to each factor and which species are responsible for the dissimilarity among the factor groups. Time is used as a factor to test for differences between sampling events.

The analysis approach used for simplified indices will depend on whether data can be transformed to meet the assumptions of parametric analyses that provide a wider range of options and greater statistical power, or whether non-parametric approaches must be used. Parametric analyses may need a data transformation such as square root, 4th root, arcsine, or $\log_{10}$ to meet the assumptions of the test. Trends are analyzed through time using a repeated measures analysis of co-variance with time as a linear variable if change appears to be occurring in a linear fashion and site as a random effect or as a repeated measures analysis of variance with a mean separation test to test for differences among time steps. However, if the assumptions of normality cannot be met using data transformations or using alternate data distributions (e.g., binomial or Poisson), then analysis can proceed using non-parametric statistics to compare two years of data (e.g., the first year against the most recent year). Potential tests include the Wilcoxon paired-sample test, signed rank test, sign test, or Statistical

of their corresponding distances (Clarke 1993). A measure of this rank-order agreement is shown as a stress value that increases with reduced dimensionality and with increasing quantity of data. Generally, non-metric multi-dimensional scaling provides a good representation of the data when stress is less than 0.20 (Clarke and Warwick 2001), though higher values do not necessarily mean a bad representation.

A cluster analysis is performed by applying the CLUSTER routine to the site dissimilarity matrix. This analysis uses group-average linking and creates a dendrogram (tree diagram) which further illustrates the hierarchical clustering among sites. Cluster delineation (circles) can be overlaid on the NMDS plot, showing similarity percentages and thus allowing the relationship between the groups to be displayed more informatively. Agreement between cluster analysis and non-metric multi-dimensional scaling strengthens support for the adequacy of both (Clarke and Warwick 2001; see Figure 14).

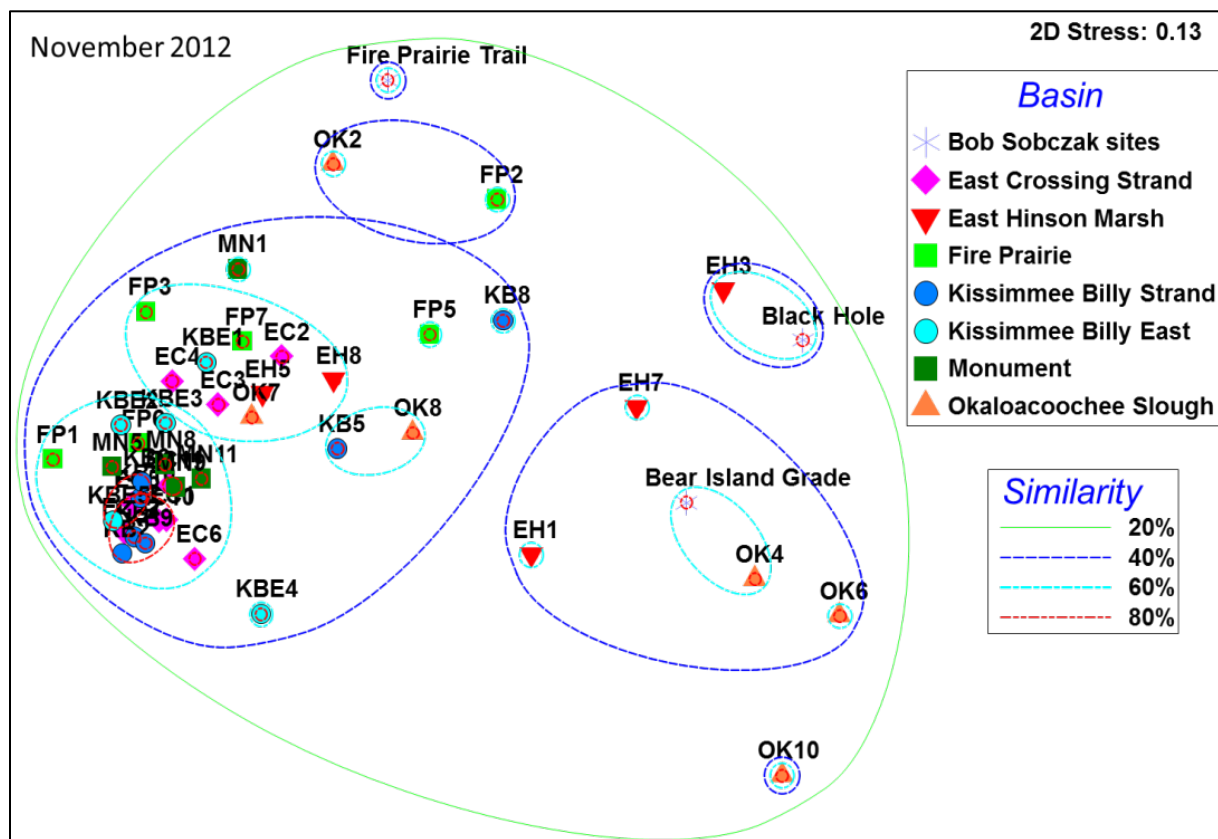


Figure 14. Example of Ordination graph created by non-metric multi-dimensional scaling (NMDS) (fourth root transformation) of diatom assemblages found in the November 2012 periphyton samples. Circles represent similarity percentages based on cluster analysis.

A two-way analysis of similarities (ANOSIM) analysis using groupings by basin and time with sites nested within basin will be used to evaluate the overarching effects of basins and time (using pairwise tests after the ANOSIM). If a time $\times$ basin interaction is suspected, analyses can also be conducted for individual basins to determine if individual basins are changing through time. A test

statistic (Global R) and a significance level (p) are generated for the analysis of similarities after 999 iterations. Global R is based on the ratio of the between-basin to within-basin dissimilarity ranking with a value that ranges from 0 (no dissimilarity) to 1 (total dissimilarity). Pair-wise comparison ANOSIM tests between sites are also calculated. The importance of the pair-wise tests is not so much the significance level (p), which can be low because of few replicates in each group, but rather the R value which provides an absolute dissimilarity measure among the samples, also on a scale of 0 to 1 (Clarke and Warwick 2001; Clarke and Gorley 2006). A similarity percentages (SIMPER) analysis is conducted for basins. Similarity percentages analysis determines which diatom species contributes the most to each basin and which species were responsible for the dissimilarities among the basins. A similar multi-year analysis can be conducted with year included as a factor.

Within Basin and Site by Site Analysis

The pilot data suggest there are already considerable differences among basin metric averages and variances, and trends will likely be different in different basins. Thus analyzing each basin separately may simplify analysis and presentation and may be necessary if an appropriate data transformation and model structure cannot be found for multi-basin analysis. In this case, sites and time will be the factors and differences measured, as described above, but by basin rather than including basin as a factor. Results are analyzed as either paired t-tests or signed rank tests within each basin. Each site is also individually assessed for whether total phosphorous levels are above thresholds. As sites are permanent, once three or more years are collected, sites are evaluated individually for trends using a regression analysis. However, analyzing change across all 44 sites together in a single analysis without including a basin effect would be inappropriate for most analyses testing for park-wide trends, as the selection probabilities differ greatly between the different basins.

Additional Analyses

The metrics and suggested analyses provided above should be viewed as a way for the South Florida/Caribbean Network to get started with reporting results. Further investigation into different indices are needed regarding how to assess change through time. For example, Gaiser (2009) uses reference sites and compares similarity and dissimilarity of all other sites with reference sites. Trexler and Gaiser (2013) use the ratio of “weedy” to endemic diatom species. Some combination of the diatom indicators in Table 9 may be most appropriate (e.g., a sum of “percent eutrophic” and “percent mesotrophic-eutrophic” diatoms). Ultimately, indices and analysis methods are needed that are statistically powerful while being communicable and meeting the needs of the National Park Service.

The results for periphyton mat TP and diatom community structure should be compared to previous results of the pilot study. If results are not significantly different, then results from the pilot study may be incorporated into long-term reporting of status and trends, where appropriate, with the date of the change in methodology clearly indicated.

Although a large proportion of the diatoms recorded in the pilot study have been identified to their eutrophic or oligotrophic indicator status, some remain unknown. After several years the indicator status of the diatoms may be elucidated from the combination of phosphorous and diatom data.

Reporting

These metrics are reported in an annual data summary report produced about six months after the South Florida/Caribbean Network receives diatom and TP data from the lab. Lab analyses typically take 6 to 12 months, so reports will be produced within 18 months of sample collection. Data summary reports will be published in the NPS Natural Resource Data Series ([Natural Resource Publication Series website](#)) if analyses, results, and interpretation stay within described procedures; or published in the Natural Resource Report Series if additional peer review is required.

The data summary report format will be cumulative, allowing each year of data to be added to the existing tables and graphs. An automated graphing procedure will be designed to allow easy update of tables and graphs to reduce the time to create annual reports. A sample data summary report is provided in SOP 13 *Data Reporting* (Whelan et al. 2019). Additional summary tables or information can be added to the summary data report as needed. Data are summarized park-wide and by basin.

This annual data summary report and resource brief will be presented to Big Cypress National Preserve managers for their review before publication. The patterns and trends documented in the report provide indicators of conditions in the park that are used as part of a comprehensive approach to resource decisions within the park. Periodic comprehensive, interpretive, peer-reviewed technical reports or scientific journal articles will also be produced at least once every five years. The data summary reports, resource brief, technical reports, scientific papers and data will also be available on the [South Florida/Caribbean Network website](#) and [irma.nps.gov](#).

Protected Data

The South Florida/Caribbean Network is not aware of any sensitive data that requires special attention in this protocol. The network will consult with Big Cypress National Preserve by February 2019 to determine if park management feels release of any information associated with this protocol requires protection from public release. Data may be protected when releasing the information will result in harm, theft, or destruction of a resource considered endangered, threatened, rare, or commercially valuable. The South Florida/Caribbean Network will restrict access to such data using an approach approved by the park. Other protected information includes personally identifiable information such as names of interns working on the protocol. These data will only be released as initials.

Metadata and Archiving

Each year the South Florida/Caribbean Network will archive the database together with associated metadata, photos, scanned datasheets, summary report, and analysis scripts on the [NPS Data Store](#). The target date for archiving all South Florida/Caribbean Network data associated with a survey year will be by the end of the following June.

Personnel Requirements and Training

Roles and Responsibilities

Periphyton collection requires considerable advance planning and scheduling to complete the necessary tasks. The following personnel are involved in implementing the periphyton monitoring protocol: SFCN Community Ecologist, SFCN biological technicians, and SFCN data manager.

The SFCN community ecologist will either act as project lead or may delegate the day-to-day project lead responsibilities to a SFCN community team member. The SFCN community ecologist retains final responsibility for implementation of the protocol and products produced.

The project lead (either the community ecologist or a designated member of the SFCN community team) is responsible for the pre-sampling planning and coordinating, sampling logistics, and post-sampling activities, as well as delegating these tasks to other staff members when necessary. Pre-sampling planning includes notification of park management and private parties, procuring needed equipment, scheduling personnel, and scheduling flights. The South Florida/Caribbean Network has cross-trained personnel so a variety of people can fulfill the mission. Training of new personnel involved in the vital sign and maintenance of physical records are responsibilities of the project lead.

A minimum of two people are required to fulfill the field portion of the Big Cypress National Preserve periphyton monitoring protocol. Additional support comes from the data management team and other personnel who assist with data processing and analysis. The participants for the field monitoring are usually South Florida/Caribbean Network personnel. However, Big Cypress National Preserve personnel may also participate in periphyton collection, providing they possess the necessary and required training for the mission. On any given mission, at least one of the paired crew members will previously have been on at least one periphyton collection event and will be able to provide guidance.

The crew must download data promptly upon their return. The project lead must ensure the data collected are entered in the database. Additional support comes from the data management team and other personnel by assisting with the processing and analyzing of data.

The project lead drafts the data summary report and the community ecologist provides oversight, assistance as needed, and reviews all products. The South Florida/Caribbean Network data manager, in cooperation with the project lead and Community Ecologist, oversees the database design, building adequate quality control procedures into the database and maintaining data security and archiving. Other South Florida/Caribbean Network personnel assist with analyses, updating the graphing template and converting the same to R scripts, and with editing of the data summary report to ensure it meets NPS Natural Resource Publications Management guidelines. The project lead works with the South Florida/Caribbean Network assistant data manager and the NPS Southeast Region I&M managing editor to update the periphyton resource brief with review by the community ecologist. The South Florida/Caribbean Network data manager is responsible for updating products on NPS Data Store and the South Florida/Caribbean Network website.

South Florida/Caribbean Network Community Ecologist

The SFCN community ecologist is responsible for ensuring the successful and safe implementation and reporting of the monitoring protocol and may either perform the project lead duties themselves or delegate them to a SFCN community team biological technician. However the SFCN community ecologist is responsible for big picture oversight of the project, reviewing the field plans, reviewing QA/QC results and ensuring all checks are complete and certifying the data, and reviewing the final data summary report. The SFCN community ecologist should have extensive training and experience in designing, conducting, and reporting ecological research preferably with experience in south Florida and with a strong interest or experience in wetland ecology or natural resources.

Qualifications and Training

As previously discussed, sites are accessed in any of the following ways: ATVs, hiking, and helicopter. Regardless of the method used to access a site, for safety and practicality, a minimum of two people are needed to perform the sample collections. This allows for one person to complete the data sheet while the other gathers samples and takes the necessary measurements. It also provides a safety partner when traveling in backcountry areas.

Personnel must thoroughly review this protocol, associated safety SOPs, and associated Job Hazard Analyses (JHAs) before implementing procedures. The project lead (community ecologist or assigned project lead) must have completed the S-372 Resource Helicopter Manager Training. At least one of the two helicopter crew members must be S-271 Helicopter Crewmember certified and have that certification up to date. Both helicopter crew members must be A-100 Basic Aviation Safety and A-107 Aviation Policy and Regulation certified and have that certification up to date.

When using ATVs to access sites, two two-person teams are used to maximize the number of samples to be collected within a given time frame. Each person that drives an ATV must be certified for ATV use and have fulfilled all the requirements necessary for ATV use in Big Cypress National Preserve. Requirements for certification include completion of the ATV Safety Institute (ASI) ATV Rider Course and the Big Cypress National Preserve Off-Road Vehicle (ORV) Operator Course, which is necessary in order for an operator permit to be issued. All ATVs used in the preserve must undergo a vehicle inspection through the Big Cypress National Preserve ORV office prior to their use in the preserve. The personnel using the ATVs are responsible for the care and maintenance of the ATVs while in the field. It is also the responsibility of the riders to ensure they have the necessary personal protective equipment, food, safety gear, and tools needed to perform the mission. Since much of the terrain is difficult to navigate, it is recommended participants have additional experience using ATVs before performing this mission.

Occasionally sites will be inaccessible by ATV because of deep water or vegetation and will require accessing on foot. The periphyton collection process does not vary and is always performed by two people. Hiking in the preserve can be physically demanding and requires the proper clothing and supplies, for the mission as well as any unexpected hardships that may arise.

Personnel must be trained in the use of park radios, GPS, YSI devices, field data sheet completion, and periphyton sample collection. The project lead and the South Florida/Caribbean Network

community ecologist will determine when new personnel have acquired proficient abilities to participate on flight or ATV missions.

At least one member on a team must have a familiarity with the major habitat types and wetland species likely to be encountered, e.g., sawgrass, muhly grass, little bluestem, beakrush, gulfdune paspalum, coastal spikerush, sagittaria spp., waterlily, cattail, and pickerelweed.

All participants in the collection process must have a basic understanding of the environment and conditions they will be exposed to. Familiarity with the instruments and tools used for gathering data is also required. When bringing a new technician into the project, it is important to provide an overview of the procedures. Simple explanations and demonstrations of the methods to remove periphyton from the collection area and store them in the collection bottles will help provide cleaner samples. At the start of each sampling season, all personnel participating will conduct sampling procedures on at least one training site together to ensure all understand the procedures and how to implement them. During this training sampling event, observers will separately collect and record field data so that sampling variability can be estimated. Then the team will discuss the results to determine how to eliminate discrepancies. Data sheets will be retained. Additionally, sample crews will always be comprised of at least one member who collected samples during the previous sampling event.

Operational Requirements

Annual Workload and Field Schedule

This protocol is implemented once a year at the beginning of the dry season (November–December). The two modes of transportation (helicopter and ATVs) used to access collection points require planning and preparation prior to any field work.

Required helicopter pre-flight activities include scheduling the flight, notifying flight following and appropriate facilities of the flight plan, packing gear, and donning flight gear. The actual flight lasts approximately five hours and is performed by two people plus the pilot. Post-flight duties include removing gear from the helicopter, completing paperwork, and stowing gear and samples for the return drive to the South Florida/Caribbean Network office. The tasks of sending post-flight paperwork, downloading pictures and GPS flight tracks onto a computer are completed upon returning to the office.

Additional time is required during the year for submitting paperwork for flight contract management. This task may take approximately 16 hours. Table 10 provides an approximate annual schedule to complement Table 11, which provides a schedule for each flight.

Table 10. Estimated annual schedule.*

Oct	Nov–Dec	Jan–Feb	Mar	6–12 months after sample submission to labs	1 month after receiving results	2 months after receiving results	3 months after receiving results
Notify BICY Fire, ORV office, Check water levels	Periphyton field sample collection	Send samples for analysis; data entry into Access database	100% verification of database by 2nd person	TP and Diatom results received and imported into database	Data set certification	Annual Data Summary Report	Update Resource Brief

* Sampling period varies and is dependent on water levels. Report production timing is based on receiving results from labs, which can take 6–12 months.

Table 11. Project time estimates per annual periphyton helicopter monitoring event. This table does not include sample processing times for diatom content and total phosphorus content.

Personnel	Tasks	Time per person (hours)	Total crew time (hours)
Project lead (1 person)	Phone calls, flight-related paperwork, notification emails to Big Cypress National Preserve Fire and Aviation	2.00	2.00
Project lead, team member (2 people)	Print data sheets, prep sample bottles, calibrate meters, upload points to GPS devices, pack and load gear	2.00	4.00
Project lead, team member (2 people)	Drive to Oasis Visitor Center in Big Cypress National Preserve	1.50	3.00
Project lead, team member (2 people)	Pre-flight – safety briefing, check certifications for pilot and helicopter, don flight gear	0.75	1.50
Project lead, team member (2 people)	Store and secure gear, perform mission	6.00	12.00
Project lead, team member (2 people)	Post flight – remove flight gear, load samples and equipment into vehicle, complete paperwork	0.75	1.50
Project lead, team member (2 people)	Return drive to South Florida/Caribbean Network office	1.50	3.00
Project lead, team member (2 people)	Fix samples, return all equipment to its proper location	0.50	1.00
Project lead or team member (1 person)	Download GPS data, download camera photos, log helicopter flight time in Excel spread sheet	1.00	1.00
Project lead or team member (1 person)	Scan data sheets, save them as PDF files, and store them in proper directory location, email Big Cypress National Preserve staff of mission completion	1.50	1.50
Project lead or team member (1 person)	Prepare and ship samples to universities for analyses, create packing list	1.50	1.50
Project lead or team member (1 person)	Enter and check field data, perform 100% Quality Assurance review	9.00	9.00
Total time	–	28.00	41.00

Follow procedures to ensure ATVs are in good operating condition prior to use (see Table 12). Performing the work in advance allows time to remedy any issues that arise.

The initial Periphyton database took approximately 120 hours to construct. The outreach program consisting of data entry, data analysis, and producing the resource brief and annual data summary

report requires approximately 100 hours to complete. The data management team spends an additional 10–20 hours throughout the year in report writing and database modifications.

Table 12. Project time estimates per annual periphyton non-helicopter (ATV/UTV, truck, hiking) monitoring event. This table does not include sample processing times for diatom content and total phosphorus content. An * indicates activities occurring over a period of three days.

Personnel	Tasks	Time per person (hours)	Total crew time (hours)
Project lead (1 person)	Check stage gauges, prepare sampling schedule and logistics, assemble necessary personnel	3.0	3.0
Project lead, team member (2 people)	Inspect ATVs (test drive, check fluids, add fuel, check batteries, check tire pressure), inspect trailer (check lights, check connections, check tire pressure)	2.0	4.0
Project lead or team member (1 person)	Contact Big Cypress National Preserve staff for additional equipment if necessary, park housing, and ORV access, complete and submit all required forms, contact Breitburn Energy personnel for notification of access	3.5	3.5
Project lead, team member (2 people)	Go through periphyton and ATV Go-No Go checklists	2.0	4.0
Project lead, team member (2 people)	Pack and load equipment into trucks and trailer, secure ATVs in trailer, fill extra fuel containers	3.0	6.0
Project lead, team members (4 people)	Buy supplies, drive to Big Cypress National Preserve Headquarters	3.0	12.0
Project lead, team members (4 people)	Complete forms for park housing, get house keys, unload equipment, prepare for sampling mission	3.0	12.0
Project lead, team members (4 people)	Truck driving, ATV driving, sample collection	33.0*	132.0*
Project lead, team members (4 people)	Fix samples, download GPS data/photos to field laptop computer, secure gear	3.0*	12.0*
Project lead, team members (4 people)	Clean house, load gear into trucks and trailer, go to Big Cypress National Preserve Headquarters to return house keys	3.0	12.0
Project lead, team members (4 people)	Return drive to South Florida/Caribbean Network office	2.0	8.0
Project lead or team member (1 person)	Download photos and GPS data to server	1.0	1.0

Table 12 (continued). Project time estimates per annual periphyton non-helicopter (ATV/UTV, truck, hiking) monitoring event. This table does not include sample processing times for diatom content and total phosphorus content. An * indicates activities occurring over a period of three days.

Personnel	Tasks	Time per person (hours)	Total crew time (hours)
Project lead, team member (2 people)	Clean ATVs and trailer, inspect for damage, conduct oil change on ATVs, clean, store, and secure gear	5.0	10.0
Project lead or team member (1 person)	Email Big Cypress National Preserve resource staff a map product showing all tracks to sites accessed during mission, scan data sheets, save them as PDF files, and store them in proper directory location	2.0	2.0
Project lead or team member (1 person)	Prepare and ship samples to universities for analyses, create packing list	1.5	1.5
Project lead or team member (1 person)	Enter and check field data, perform 100% Quality Assurance review	9.0	9.0
Total time	–	79.0	232.0

Facility and Equipment Needs

To accommodate this monitoring protocol, the facility needs to have ample refrigerated storage for collected samples and space for ATVs, ATV trailer, and field gear. Appropriate personal protective equipment (PPE) must be provided and other safety requirements need to be considered in advance.

Flights are currently conducted using an NPS helicopter and pilot. The helicopter is located at a hangar in Big Cypress National Preserve. Arrangements can be made using a contractor (budgeting and contracting permitting) if NPS equipment and personnel are not available. Appropriate flight gear (helmet, leather boots, flight suit, and gloves), camera, GPS units, notebook, and data sheets are required for each flight. A South Florida/Caribbean Network vehicle (for the drive to and from the airport) is reserved on the calendar in advance of the flight date.

Startup Costs and Budget Considerations

Startup costs consist of the equipment mentioned in the SOPs. It includes a handheld VHF radio, GPS, flight gear, water-quality meters, and ATV equipment. This equipment costs approximately \$18,500 (Table 13). The A-100, A-107, and S-271 flight training courses and ATV courses are currently provided at no charge, but travel expenses may need to be added depending on the training location. Helicopter costs are currently covered by Big Cypress National Preserve Resource Management, therefore only the salary of the flight crew should be factored in. Budget constraints may limit the quantity of trips. The combination of data processing, data management, and reporting equals approximately 30% of the budget. One-time startup costs are detailed in Table 13, and repeating annual costs are outlined in Table 14.

Table 13. One-time costs for periphyton monitoring operations. These costs are approximate based upon 2017 estimates. Costs of vehicles, computers, and standard office productivity software, etc. are not included. An asterisk (*) indicates equipment also used in other vital sign monitoring.

Equipment	Cost per unit	Quantity	Total crew cost
Nomex Flight Gloves*	\$ 24.00	2	\$ 48.00
Nomex Flight Suit*	\$ 142.00	2	\$ 284.00
Flight Helmet*	\$ 726.00	2	\$ 1,452.00
Flight Helmet Bag*	\$ 16.00	2	\$ 32.00
Leather Boots (for ATV and helicopter)*	\$ 100.00	4	\$ 400.00
Garmin GPS60CSx or similar handheld unit*	\$ 350.00	2	\$ 700.00
Garmin GPSMAP 640 or similar*	\$ 650.00	1	\$ 650.00
YSI water-quality meter*	\$ 1,000.00	2	\$ 2,000.00
Olympus Stylus Tough waterproof camera	\$ 250.00	2	\$ 500.00
ATV	\$7,832.45	2	\$ 15,664.90
ATV Trailer	\$4,164.95	1	\$ 4,164.95
ATV Spare Tires	\$ 200.00	1	\$ 200.00
ATV Helmet	\$ 55.00	4	\$ 220.00
ATV Goggles	\$ 42.00	4	\$ 168.00
ATV Gloves	\$ 20.00	4	\$ 80.00
BK DPHX5102X Radio*	\$ 2,035.00	2	\$ 4,070.00
ACR ResQLink Personal Locator Beacon*	\$ 290.00	2	\$ 580.00
Primer	\$600.00	1	\$600.00
Total charges	\$18,497.40	—	\$31,813.85

Table 14. Yearly cost estimates for periphyton operations. These costs are approximate based upon 2017 salary estimates and helicopter costs.

Expense	Estimated time	Estimated annual cost
Salaries for mission prep (ATV and flight)	25 hours	\$1,125
Approximate salaries for sample collection (ATV and flight)	320 hours	\$14,400

Table 14 (continued). Yearly cost estimates for periphyton operations. These costs are approximate based upon 2017 salary estimates and helicopter costs.

Expense	Estimated time	Estimated annual cost
Housing costs	4 days /2 people per room (120.00/ day)/ room	\$960
MSU sample processing	Single payment	\$14,700
FIU sample processing	Single payment	\$3,100
Report writing and analysis	100 hours	\$4,500
Additional data management support	20 hours	\$1,000
YSI calibration	—	\$250
Sample bottles	—	\$100
Formalin	—	\$100
Shipping of samples	—	\$50
Totals	—	\$40,285

Software

Software needs include statistical software (R, Primer), Microsoft Access, and a word processor such as Microsoft Word.

Safety

When a helicopter or an ATV cannot get you to a collection site, you may have to hike in. Wear the appropriate attire for hiking such as closed-toed shoes or hiking boots and long pants. Bug repellent is often necessary too, especially during summer months when biting insects are at their peak.

The number of people required to collect samples differs between access methods. Regardless of the method used to access a site, for safety and practicality, a minimum of two people are needed to perform the sample collections. A first aid kit is kept readily available.

Following are more details on the training and safety requirements for helicopter use, ATV use, and use of formalin to fix diatom samples.

Helicopter Use

Flying in helicopters requires considerable safety training followed by mission specific caution and care. All personnel are responsible for following the safety rules, regulations, requirements, policies, and procedures of the Department of Interior (DOI), as well as of the park in which they are working. A culture of safety and operational leadership must prevail in which problems are considered proactively and each person must feel empowered to think about potential problems, and speak up

early about safety concerns regardless of their position. The rest of the flight team and their supervisor must address all safety concerns seriously and respectfully.

All DOI personnel participating in helicopter flights for this monitoring project are required to complete the following helicopter training courses every three years:

- A-107, Aviation Policy and Regulations I (online).
- A-100, Basic Aviation Safety.
- S-271, Helicopter Crewmember (at least one crewmember).
- S-372, Resource Helicopter Manager (for the project lead).

All personnel must read and follow:

- Standard Operating Procedure 2 *Helicopter and ATV/UTV Procedures for Periphyton Sampling* (Muxo 2019a).
- Project Aviation Safety Plan Big Cypress Preserve: Big Cypress National Preserve, located on the SFCN server at: Z:\Helicopter\Safety_plans\Periphyton.
- Helicopter Job Hazard Analyses, located on the SFCN server at: Z:\SAFETY\SFCN_SOPs_with_JHAs\JHAs\Community Group JHA's\Job_Hazard_Analysis_Periphyton\

Other pertinent forms are located in the Periphyton supplemental forms folder, which can be found at: Z:\SFCN\Vital_Signs\Periphyton\documents\protocol\Latest_version\Supplemental_Material. The folder contains the following forms.

- SFCN_Float_Plan.doc.
- Flight_Request_Form_9400_blank_use_this.pdf.
- SFCN Helicopter Go No Go Checklist.
- Aviation_Risk_Assessment.docx.
- Hazardous_Materials_Manifest.docx.

These forms may be updated after publication and thus should be seen only as examples to help personnel locate the correct forms.

ATV Use

Proper training in ATV use is required for accessing collection sites via ATV. Successful completion of the ATV Safety Institute “ATV Rider Course” is mandatory prior to using an ATV within the South Florida/Caribbean Network. The course provides instructions for risk management, safe maneuvering, maintenance, and personal protective equipment (PPEs). All required personal protective equipment must be worn during ATV use. Training in conjunction with safety gear mitigates inherent dangers associated with ATV use. The required PPE for ATV use include helmet, eye protection, gloves, boots, and minimum clothing of long-sleeved shirt and long pants.

For more detailed information regarding the operation and safe use of ATVs, please see the ATV Job Hazard Analysis (JHA) and the South Florida/Caribbean Network ATV Go/No Go Checklist. The most recent versions of the JHA and Go/No Go Checklist are found on the SFCN server at: Z:\SAFETY\SFCN_SOPs_with_JHAs\JHAs\Community Group JHA's\JHA_ATV and Z:\SFCN\General_SOPs\SFCN ATV Go_No_Go respectively.

Basic tools are stored in each ATV cargo box in the event of mechanical issues. Spare tires are available in the trailer.

Formalin Use

This protocol uses 3% formalin to fix and preserve the diatom samples so they can be sent for laboratory processing. Three percent formalin solution is diluted from either 37% formaldehyde or 10% formalin solution. Formaldehyde and 10% formalin solutions are stored at Everglades National Park and dilutions must occur under a chemical fume hood. All personnel must read and follow procedures, job hazard analyses, and the following related safety information;

- Standard Operating Procedure 14 *Shipping of Formalin Solutions* (Muxo 2019b).
- Standard Operating Procedure 17 *Use of Formalin in Field and Laboratory Settings* (Urgelles 2019b).
- South Florida/Caribbean Network Job Hazard Analysis (JHA) “Use of formalin for the preservation of periphyton mat samples”
- Material Safety Data Sheets (MSDS) for Formaldehyde 37% solution and Formalin 10% buffered solution
- NPS Occupational Safety and Health Program, Reference Manual 50B, Section 4 Occupational Health
- OSHA Formaldehyde Standard 29 CFR, Occupational Safety and Health Standards - 1910, Toxic and Hazardous Substances - 1910.1048, Formaldehyde – Appendices A, B, C, D, & E

Procedures for Revising the Protocol

As time progresses and monitoring techniques evolve, revisions to the protocol narrative and SOPs will be necessary. Once published in the Natural Resource Report Series, the *Periphyton Monitoring in Big Cypress National Preserve: Protocol Narrative* can only be modified through the use of a versioning system and guidance as described in SOP 12 *Revising the Protocol* (Muxo 2019c). Changes are designated as minor (e.g., software update or technology change) or major (e.g., changes in field data collection methods or expanding to new areas of Big Cypress National Preserve). Major changes in SOPs that affect the interpretation of the data may trigger an update to the protocol main document and corresponding change log. Minor changes to SOPs are required to be reviewed by the network program manager. Any changes to the protocol narrative and major changes in SOPs are referred to the regional program manager who will determine if additional scientific review is warranted. The Big Cypress National Preserve staff will be kept informed of all major changes to the protocol and asked to review them when appropriate (e.g., changes to scope, sampling design and frequency, reporting, or safety).

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Appendix A. Use of Diatom Assemblages to Assess Periphyton Quality in Big Cypress National Preserve

Urgelles, R. 2017. Use of Diatom Assemblages to Assess Periphyton Quality in Big Cypress National Preserve. South Florida/Caribbean Network. Palmetto Bay, Florida.

Periphyton is an algal matrix dominated by calcium-precipitating cyanobacteria, green algae, and diatoms. South Florida wetlands such as the Florida Everglades and the Big Cypress Swamp are described as oligotrophic (nutrient-poor, oxygen-rich) and phosphorus-limited ecosystems. Numerous periphyton studies in the Florida Everglades have shown that at phosphorus concentrations above 10 µg/L (10 parts per billion [ppb]), both diatom and soft-algae (non-diatom) communities in the periphyton mats abruptly shift in composition (McCormick et al. 1996; McCormick and O'Dell 1996). This change in algal composition can increase the risk of losing the floating calcareous periphyton mats characteristic of oligotrophic environments, subsequently producing cascading effects throughout the ecosystem's food web (Gaiser et al. 2005). Due to this strong relationship between algal community composition and phosphorus levels, both diatom and soft-algae assemblages make excellent bio-assessment tools for measuring nutrient impacts on the aquatic ecosystem of Big Cypress National Preserve (BICY).

There were two main objectives during the first pilot year of sampling for the periphyton vital sign (December 2008) in the northwest section of the preserve. First, to characterize the diatom and soft-algal assemblages found in periphyton from sites predicted to be in a natural or impacted state, based on water quality data from the nearby water quality station. And, determine if there is sufficient assemblage differentiation between these sites to use periphyton as a long-term indicator of ecological impact. The second objective was to determine if both diatom and soft-algal metrics were needed or if diatoms alone would suffice. This assemblage characterization for both algal groups at each site was deemed important to determine the relative value of both types of assays in future periphyton assessments. Changes in diatom species composition have long been used to assess changes in water quality because of the accuracy with which species can be identified and sensitivity of species to changes in environmental conditions (Stevenson et al. 2010). However, soft algae are relatively imprecise indicators of environmental changes because of challenges associated with species identification and characterizing the relative abundances of species (e.g. the use of natural units as opposed to individual cells).

In December 2008, 41 periphyton samples were collected from nutrient impacted and unimpacted hydrological basins (impacted basins are Bear Island, BI Slough, EH Marsh, and OK Slough; unimpacted basins are EC Strand, Monument, Fire Prairie, and Monument Trail; Figure A-1). Three replicate samples were taken near long-term water-quality monitoring stations. The pilot study samples included 17 paired samples collected from cypress domes and nearby graminoid communities. Sites were selected based on access via ATV and four-wheel drive vehicle.

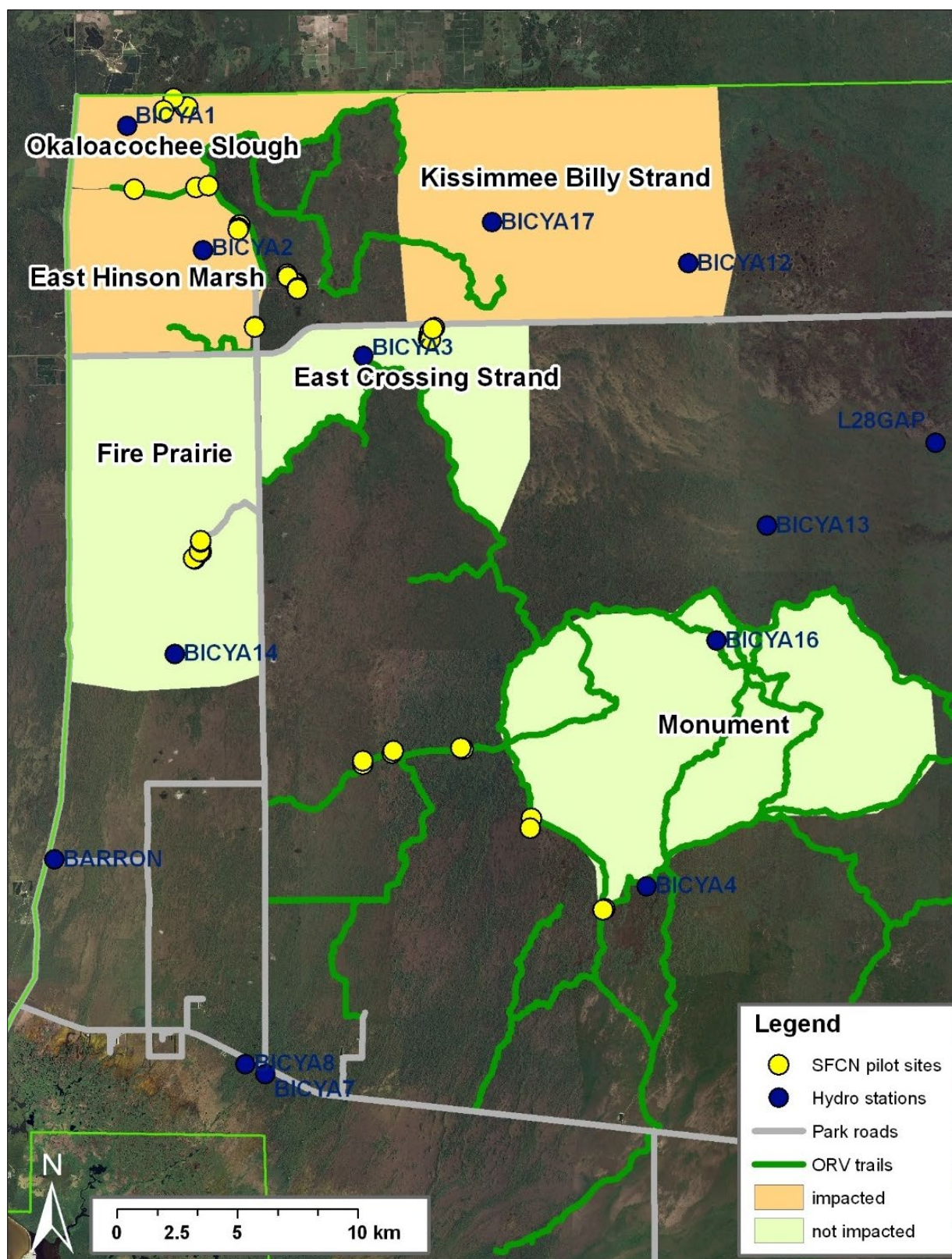


Figure A-1. Sites of periphyton pilot study sampling in Big Cypress National Preserve. Impacted basins are colored in orange and unimpacted basins are colored in green, December 2008.

The periphyton samples were sent to Dr. R. Jan Stevenson at Michigan State University for processing. Each sample was divided into two subsamples for diatom and soft-algae analyses. Data for diatom and soft-algal species were grouped into impacted and natural state (unimpacted) sites and tested for significant differences using the Multi-Response Permutation Procedure (MRPP) function in the R statistical software. The MRPP analyses showed a significant difference in diatom community composition between impacted and unimpacted sites ($p = 0.00049$) and also a significant difference in soft-algal communities between impacted and unimpacted sites ($p = 0.001$). Diatom and soft-algae data were also analyzed using PRIMER v.6.1.16 statistical software. Diatom and soft-algae assemblage dissimilarities between impacted and unimpacted sites were again found to be significant (Figure A-2; ANOSIM statistical test, $p = 0.001$ for both).

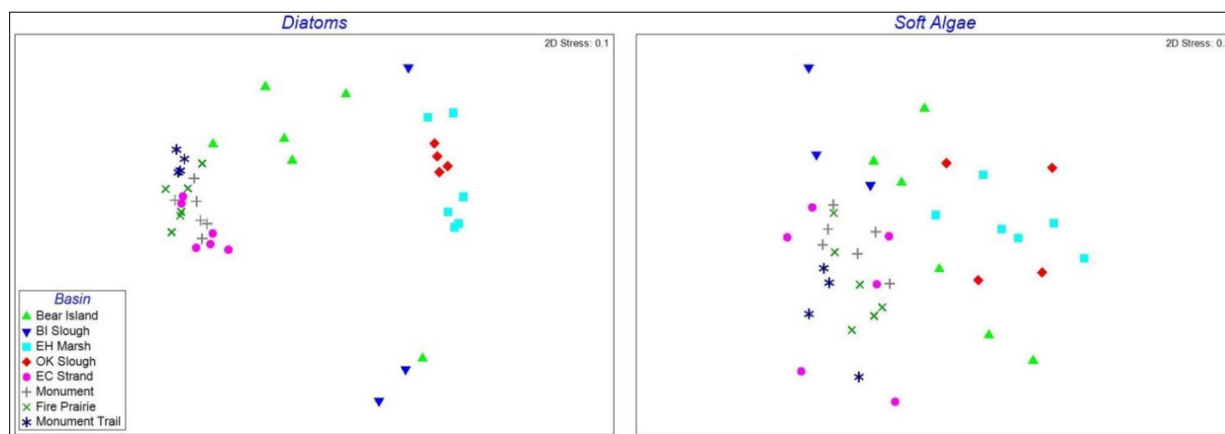


Figure A-2. Non-metric Multi-Dimensional Scaling (NMDS) graphs showing site dissimilarities for diatom communities (left) and soft-algae communities (right), created with PRIMER v.6.1.16 statistical software. Both diatom and soft-algae samples were standardized by totals followed by a square root transformation. Impacted basins are Bear Island, BI Slough, EH Marsh, and OK Slough. Unimpacted basins are EC Strand, Monument, Fire Prairie, and Monument Trail.

However, site dissimilarity was found to be statistically greater when based on diatom community structure (Global $R = 0.899$) than when based on soft-algae community structure (Global $R = 0.668$). When illustrated as an NMDS graph, diatom dissimilarities appear as clearly marked and pronounced separations between impacted and unimpacted sites (Figure A-2). Zalack and Stevenson (2010) recommended the use of diatoms for analyses. They state that assessments of diatoms would provide similar results as assessments with both diatom and non-diatom algae. This would save funds and allow the use of more developed taxonomic information and existing diatom-based metrics. Thus, the South Florida/Caribbean Network concluded that assessments of general ecological conditions of wetlands in Big Cypress National Preserve with diatoms alone would provide a similar amount of information as assessments with diatoms and soft algae, thereby allowing analysis of a larger number of samples for the same analytical costs. Diatom metrics have been developed much more thoroughly in the literature than metrics using soft algae and identification of diatom species is much more precise. Even though soft algae have considerable ecological importance in the Everglades, soft algae

provide little additional information about water quality conditions than just using diatoms (Zalack and Stevenson 2010).

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Appendix B. Justification for Habitat and Site Selection for Periphyton Collection in the Big Cypress National Preserve

Whelan, K. R. T., and R. Urgelles. 2017. Justification for habitat and site selection for periphyton collection in the Big Cypress National Preserve. South Florida/Caribbean Network. National Park Service, Palmetto Bay, Florida.

The Big Cypress National Preserve (BICY) encompasses a mosaic of diverse vegetation communities including pine flatwoods and hardwood hammocks in the upland areas, and cypress domes, wet prairies, and marshes in areas of low relief. Hydrology is the major environmental driver of the low-elevation wetland communities. The quantity and quality of water delivered to these wetlands can alter plant communities and their associated animal assemblages. Numerous sloughs throughout the preserve allow for the transport of water from one end of the preserve to the other. However, artificial barriers and water management restrict and delay the flow of water to the wetlands. Altered hydrological flow, coupled with poor water quality due to nutrient enrichment, represents an undesirable condition that will not only create changes in wetland community structure (both floral and faunal), but will allow for the introduction and expansion of invasive or exotic species. Of particular concern is the northwest section of the preserve, which has been subjected to pollutants from upstream development and agricultural runoff. Water-quality stations in northwest Big Cypress National Preserve have recorded high total-phosphorus (TP) concentrations in the water column. Changes of this magnitude have the potential to impact neighboring terrestrial communities. Detecting an early response to altered hydrology is vital to prevent widespread changes throughout the preserve.

Periphyton is an important basal component of aquatic food webs and is used as an early-warning indicator of changes in water quality. Periphyton is abundant in the marshes of the preserve and is comprised of diatoms, soft algae, cyanobacteria, micro-invertebrates and detritus. Assessment of periphyton communities, such as diatoms, can be used to evaluate wetland ecosystem health and integrity.

Calcareous periphyton mats grow best in low-nutrient and high-light settings, hence the open graminoid marshes in the preserve. However, for logistical planning, it is best to sample graminoid marsh sites adjacent to broadleaf marsh areas as the latter retain water for a longer period of time due to their lower elevation, thus allowing access to the community into the beginning of the dry season when helicopter, ATV, swamp buggy, and walking are more practical.

In December 2008, the South Florida/Caribbean Network (SFCN) initiated pilot sampling for periphyton in the northwest portion of Big Cypress National Preserve. Among the samples collected, there were several from sites near the water-monitoring stations that were reporting high TP content in the water column as well as near those reporting normal background levels of TP. Samples were processed to determine the diatom and soft algae community composition and abundance. Diatom and soft algae species from these sites were compared to indicator species from other studies.

At the beginning of the SFCN periphyton monitoring, there had been limited periphyton sampling in the preserve. During our pilot work we identified a number of concerns and wanted to address the following questions:

1. Are there differences between diatom assemblages in cypress domes and those in immediately adjacent graminoid or broadleaf marshes?
2. Are there differences between soft algae and diatom communities when used as indicators of water quality impacts?
3. What is the distance within which samples can be considered part of the same “site”?
4. What is the time frame represented by a periphyton sample?

Questions 1 and 2: Graminoid Marsh vs. Cypress Dome and Diatoms vs. Soft Algae

Pilot sampling was conducted from December 8–10, 2008. Sampling at each site took place in two community-types: cypress domes (dome) and graminoid marshes (marsh). In general, samples were paired but at some locations both habitat types were not present. A goal of the sampling was to determine if community structure differed significantly between diatom or soft algae assemblages found in cypress domes and those found in immediately adjacent marshes as it related to determining if the sample was from an impacted or unimpacted basin.

The 41 periphyton samples collected in 2008 were sent to Dr. R. Jan Stevenson at Michigan State University (MSU) for processing (Figure B-1; impacted basins are Bear Island, BI Slough, EH Marsh, and OK Slough. Unimpacted basins are EC Strand, Monument, Fire Prairie, and Monument Trail). The Stevenson lab provided results of their analyses on the diatom communities found in the 2008 periphyton samples (Zalack and Stevenson 2010). The South Florida/Caribbean Network reviewed the recommendations and then investigated the diatom and soft algae data. The species composition did not differ greatly between dome and marsh communities and the results indicated that these habitat differences did not affect the ability to identify if a site was in an impacted or unimpacted state (Figure B-2).

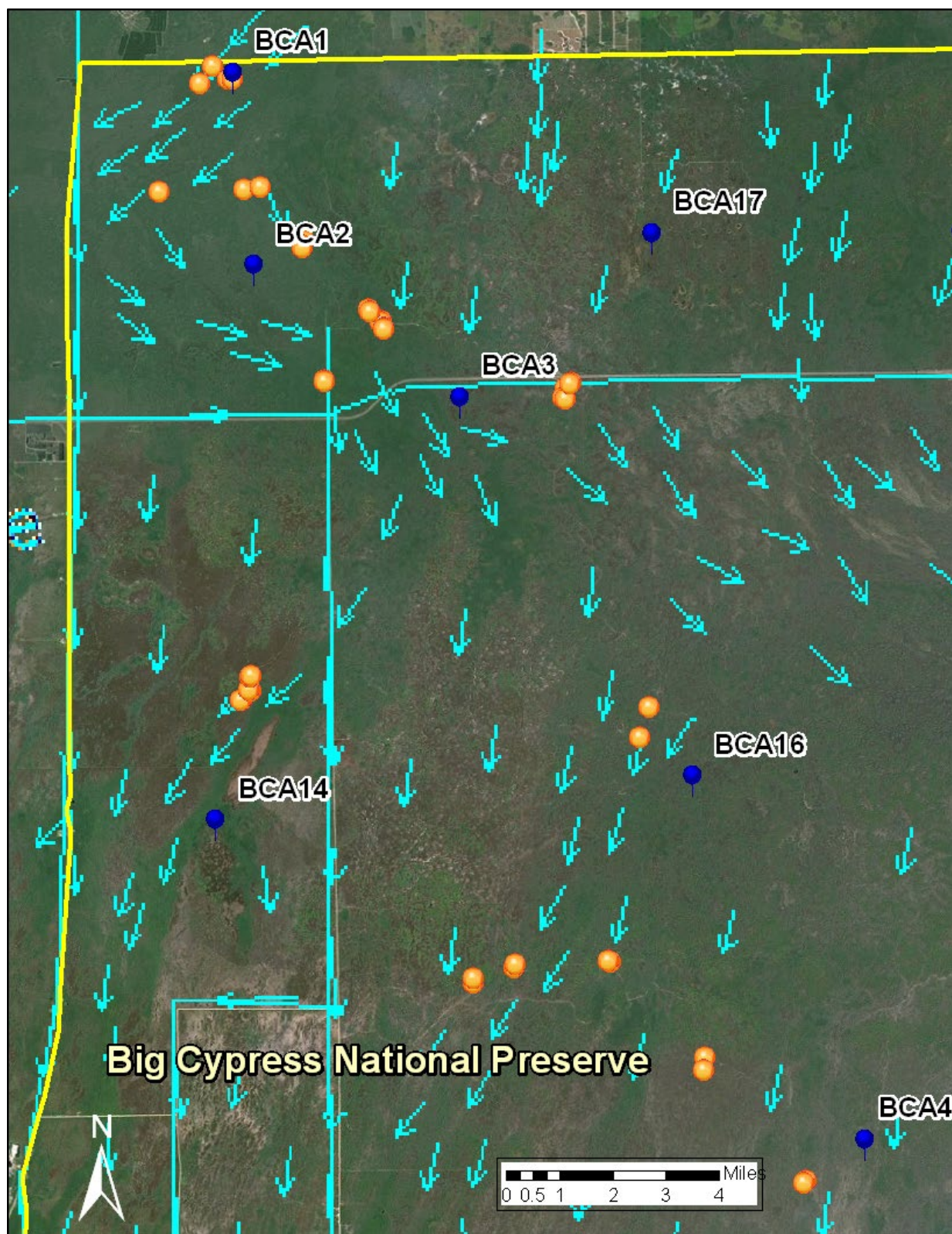


Figure B-1. Periphyton collection locations from pilot sampling, December 2008 (orange dots). Big Cypress National Preserve water quality sites (blue dots). Cyan arrows represent hypothetical water flow directions based on Klein (1970).

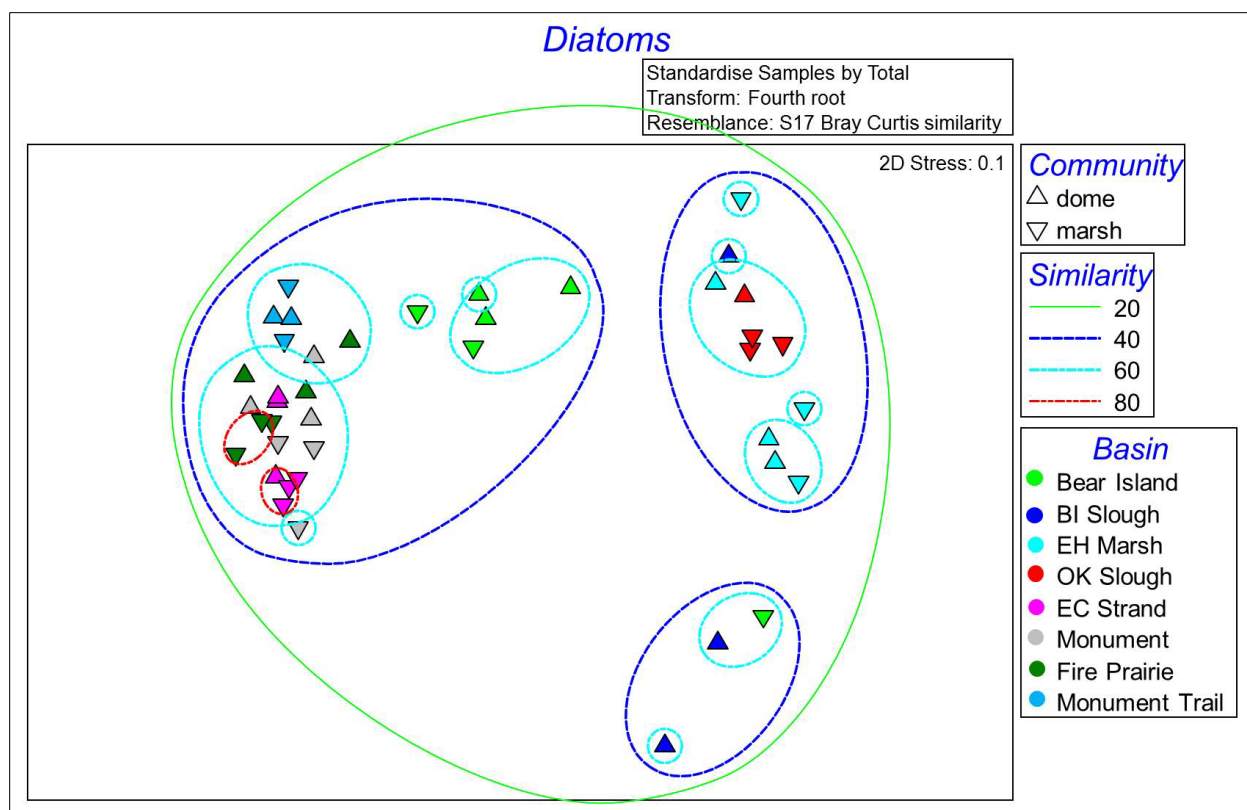


Figure B-2. Non-Metric Multi-Dimensional Scaling of diatom communities in periphyton mats from initial pilot sampling, December 2008 (fourth root transformation). Circles indicate percent similarity of diatom communities between collection sites. Impacted basins are Bear Island, BI Slough, EH Marsh, and OK Slough. Unimpacted basins are EC Strand, Monument, Fire Prairie, and Monument Trail.

Analyzing the results from the perspective of trophic category, a paired t-test and a signed rank test were conducted on the differences in percent oligotrophic diatoms and the differences in the percent eutrophic diatoms between domes and marshes at the 17 sites with paired samples. Neither test showed a significant difference in either metric between domes and marshes (Table B-1).

Table B-1. Paired samples of dome and marsh periphyton composition by trophic category compared using a paired t test and Wilcoxon Signed Rank test.

Current Site Name – Original Name	% Eutrophic Diatoms Dome	% Eutrophic Diatoms Marsh	% Oligotrophic Diatoms Dome	% Oligotrophic Diatoms Marsh	% Eutrophic Difference (Domes – Marsh)	% Oligotrophic Difference (Domes – Marsh)
EC11 - Alley 1	1%	0%	54%	26%	0.007	0.283
EC11 - Alley 2	2%	5%	58%	43%	-0.032	0.143
EC11 - Alley 3	9%	1%	48%	31%	0.083	0.172

Table B-1 (continued). Paired samples of dome and marsh periphyton composition by trophic category compared using a paired t test and Wilcoxon Signed Rank test.

Current Site Name – Original Name	% Eutrophic Diatoms Dome	% Eutrophic Diatoms Marsh	% Oligotrophic Diatoms Dome	% Oligotrophic Diatoms Marsh	% Eutrophic Difference (Domes – Marsh)	% Oligotrophic Difference (Domes – Marsh)
BI1 – Bear Island 1	42%	93%	7%	0%	-0.512	0.072
BI2 – Bear Island 2	56%	38%	9%	10%	0.187	-0.013
BI3 – Bear Island 3	9%	26%	2%	9%	-0.168	-0.070
MN12 – Concho Billy 1	3%	2%	24%	25%	0.015	-0.002
MN13 – Concho Billy 2	9%	3%	46%	29%	0.058	0.165
MN14 – Concho Billy 3	11%	3%	34%	49%	0.080	-0.155
EH3 - EH 1	77%	63%	0%	0%	0.138	0.000
EH3 - EH 2	53%	79%	0%	0%	-0.262	0.002
EH11 - EH 3	50%	78%	0%	0%	-0.283	0.000
FP6 - Fire 1	7%	9%	28%	42%	-0.015	-0.138
FP6 - Fire 2	6%	5%	29%	36%	0.010	-0.077
FP6 - Fire 3	7%	3%	58%	50%	0.038	0.078
MT1 – Monument N	13%	5%	48%	39%	0.082	0.098
MT2 – Monument S	10%	11%	38%	36%	-0.007	0.027
All Sites Average	21%	25%	28%	25%	-3%	3%
All Sties Standard Deviation	24%	32%	22%	18%	18%	12%
All Sites Number of pairs (n)	17	17	17	17	17	17

Table B-1 (continued). Paired samples of dome and marsh periphyton composition by trophic category compared using a paired t test and Wilcoxon Signed Rank test.

Current Site Name – Original Name	% Eutrophic Diatoms Dome	% Eutrophic Diatoms Marsh	% Oligotrophic Diatoms Dome	% Oligotrophic Diatoms Marsh	% Eutrophic Difference (Domes – Marsh)	% Oligotrophic Difference (Domes – Marsh)
All Sites	6%	8%	5%	4%	4%	3%
Standard Error						
All Sites t value	–	–	–	–	-0.7961	1.2292
All Sites Prob>t	–	–	–	-	0.4376	0.2368
All Sites Wilcoxon Signed Rank Test	–	–	–	-	Prob>0.25	Prob>0.25

The Multi-Response Permutation Procedure (MRPP) in the R statistical program was not able to distinguish diatom or soft algal communities from marsh and domes ($p > 0.05$) among all sites. Diatom communities from unimpacted sites did not differ significantly between marshes and domes ($p > 0.05$) nor did soft-algal communities ($p > 0.05$). Within impacted sites there was no significant difference in diatoms or soft algae between domes and marshes ($p > 0.05$, $p > 0.05$, Zalack and Stevenson 2010). Zalack and Stevenson (2010) concluded that the strongest factor affecting species composition within the 2008 dataset was anthropogenic (assumed to be phosphorous based upon other Everglades research by McCormick and O'Dell [1996]). Using a number of multiple statistical analyses (MRPP, NMDS and indicator species analysis) they were able to distinguish between unimpacted and impacted sites.

When the diatom and soft algae data were graphically visualized in a Non-metric Multidimensional Scaling (NMDS) plot (Figure B-3), for the diatoms the NMDS ordination well represented the samples (assessed from 2D stress of 0.1) where as for the soft algae the NMDS ordination needed to be interpreted with more caution (stress 0.2; Clarke and Warwick 2001). These results indicated distinct diatom assemblages in impacted and unimpacted sites within northwest Big Cypress National Preserve, and suggested that diatoms were a clearer indicator of site category than soft algae although the general results were similar. We believe that examination of the diatom composition of marsh communities alone will provide sufficient information to determine if a site is in an impacted or unimpacted state. In addition to the aforementioned findings, there are several reasons to sample diatoms in marsh habitat. These reasons include: (a) expansive literature exists on diatoms' use for water quality assessments, (b) recommendation from Dr. Jan Stevenson (Zalack and Stevenson 2010) a specialist in this field, (c) compatibility to Everglades' monitoring, and (d) lab analysis is less expensive for diatom samples than processing soft algae samples.

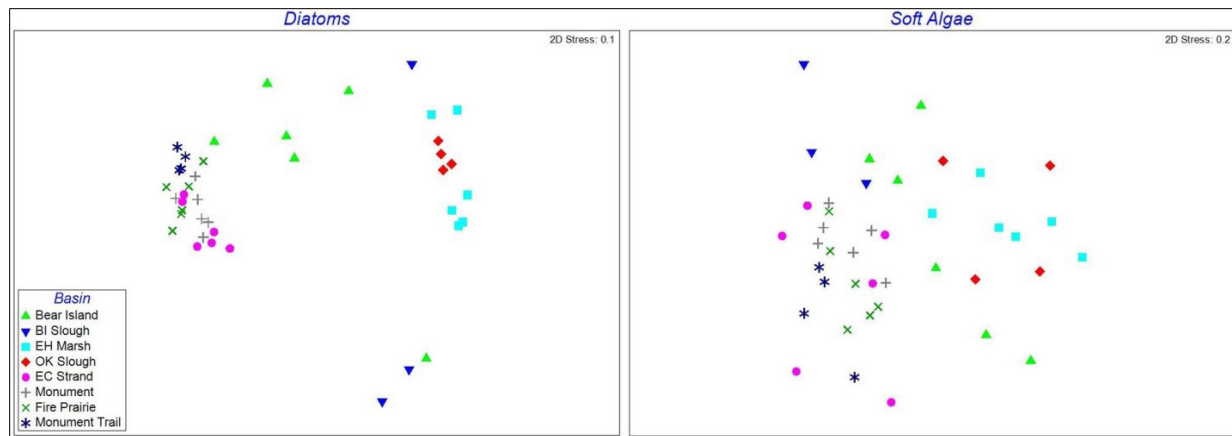


Figure B-3. Non-metric Multidimensional Scaling (NMDS) graphs showing site dissimilarities for diatom communities (left) and soft-algae communities (right), created with PRIMER v.6.1.16 statistical software. Both diatoms and soft-algae samples were standardized by totals followed by a square root transformation.

Question 3: Distance within Which Samples can be Considered Part of the Same “Site”

During the marsh dry-down period, the exact location of the water line and the corresponding floating periphyton mat varies with each sample visit. Thus, even though sites are permanent, the specific sample location can vary from year to year. It is also dependent on a safe helicopter-landing location, which is dependent on wind and water levels. Knowing the distance within which samples are part of the same “site” is important for the safe and logistically feasible implementation of this protocol.

The South Florida/Caribbean Network used pilot data from 2010–2013 to investigate the relationship of distance between sample collection locations within a site and the similarity of the diatom community composition in the samples. During sampling, the network did not return to the same exact GPS location each year, but instead returned to the larger site. An aerial view of a typical periphyton sampling site gives you an appreciation of the relative relationship of the broadleaf depressional marsh associated with the graminoid prairie (Figure B-4). Each year only one sample was collected at the site. As the year-to-year variability in diatom-community species composition was low in the more oligotrophic sites, the network chose to use samples collected from these sites with multiple samplings between 2010 and 2013, and ignored the time effect in order to evaluate the relationship in distance between the sample locations at a single site and similarities of the diatom communities.

Similarities matrices were calculated using diatom species count data for each site and each year between all samples collected. Within each site we determined the greatest distance between sample locations and the corresponding similarity between those specific sample pairs. Similarity matrices were calculated in PRIMER v.6.1.16 (PRIMER-E Ltd., Plymouth, UK).

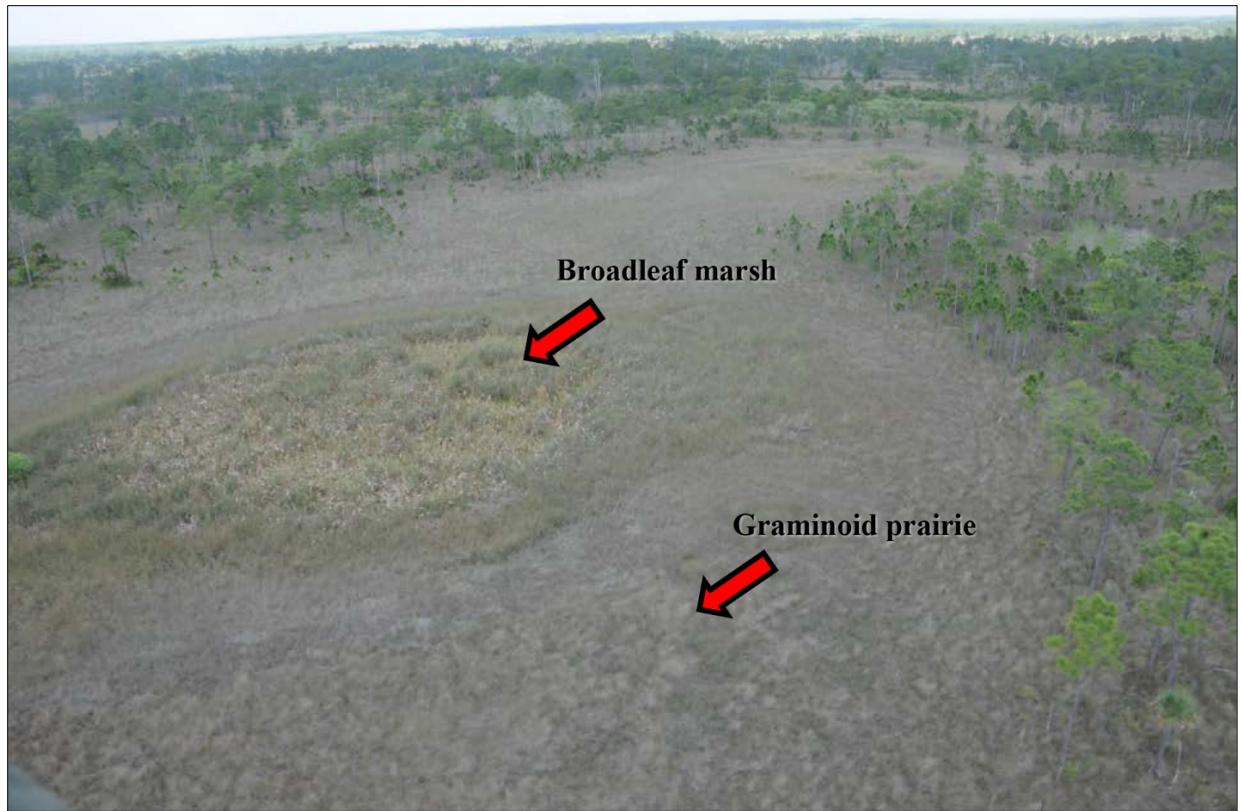


Figure B-4. An aerial view of a typical periphyton sampling location in Big Cypress National Preserve, showing broadleaf marsh surrounded by graminoid prairie.

The diatom data was non-normally distributed because many samples lacked specimens of one or more species, as is common with abundance and count data. The data was standardized to reflect relative densities of species for each site and subsequently applied a double square-root (4th root) transformation to better represent all species in the samples (Clarke and Warwick 2001). The standardized and transformed data were used to generate a sample similarity matrix using a Bray-Curtis similarity coefficient (δ) (values ranging from 0 (no similarity) to 100 (total similarity) between each pair of site means (Faith et al. 1987; Clarke and Warwick 2001). The site-similarity matrix was used to perform CLUSTER and SIMPROF analyses.

We used ArcMap 10.3 and generated a proximity analysis using the Near Table function to determine linear distance between sample locations. Table B-2 shows the sites that had the greatest linear distances over all the sample events and the similarity associated with that sampling as well as the similarity that would be considered significantly different. Out of the ten sites evaluated, only two sites had similarity below the corresponding threshold. The difference was minimal and EC10 actually had the smallest distance among samples of the 10 sites. Figure B-5 further demonstrates there is no relationship between distance and similarity score and a correlation between the two was not significant at a distance up to 320 meters (1,050 feet [ft]).

Table B-2. Pilot study site with maximum distance among the multiyear samples and corresponding similarity. The percent similarity for each site that is the cutoff for determining if a site is significantly different is also provided.

Site	Max distance (m)	Corresponding similarity	Year to year	Percent similarities greater than XX were not significantly different
KB1	321	61.63	2010 to 2011	54
EC1	274	68.43	2010 to 2012	61
FP7	185	67.01	2011 to 2012	54
EC5	172	69.67	2010 to 2013	62
KB5	117	65.63	2012 to 2013	43
KB6	98	61.97	2010 to 2012	58
KB2	95	51.05	2010 to 2012	58
MN8	70	59.12	2011 to 2012	58
MN9	45	73.32	2012 to 2013	62
EC10	30	61.70	2010 to 2011	63

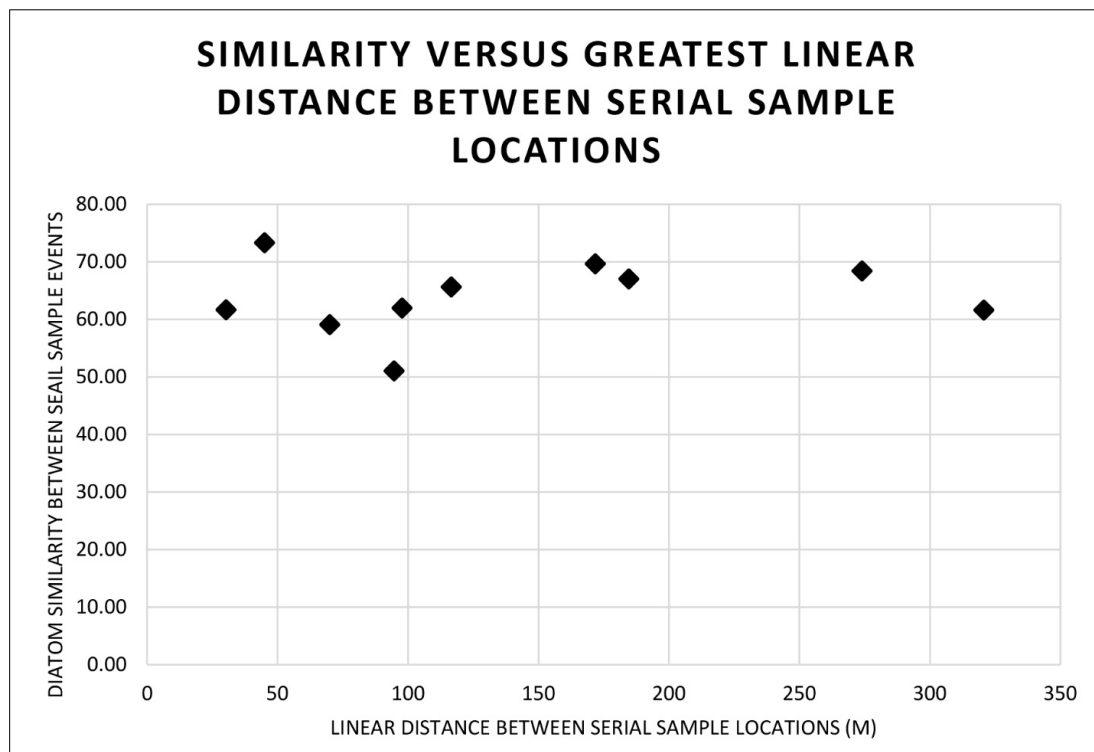


Figure B-5. Diatom community similarity versus the greatest linear distance between serial sample locations.

The results suggest that diatom similarity in different years does not have a linear relationship with distance at least up to collection locations that are up to 320 meters (1,050 ft) apart. These findings are similar to the periphyton monitoring program from Everglades Restoration (RECOVER monitoring program Evelyn Gaiser, Florida International University, personal communication, 2017). The Everglades monitoring program assumes that sample locations that are up to 500 meters (1,640 ft) apart all represent one site. Their sampling results indicated that there was greater similarity within collection locations that were within 500 meters (1,640 ft) across sample years than at greater distance within the same sample year (RECOVER monitoring program, Evelyn Gaiser, Florida International University, personal communication, 2017).

Question 4: Time Represented by a Periphyton Sample

In contrast to water-quality sampling which represents an instantaneous snapshot in time, periphyton community composition is integrative of a time period previous to the sampling event. Periphyton collection occurs at the end of the wet season. The sampling of the periphyton is timed to represent the peak water infiltration into the preserve for outside water inputs. This in flow of water and associated nutrients from outside sources occurs at the end of the wet season during the peak high water (Robert Sobczak, hydrologist Big Cypress National Preserve, personal communication, 2007). The Big Cypress National Preserve water-quality sampling program has bi-monthly water collections during this time period to capture the water quality associated with the peak high-water events (Figure B-6).

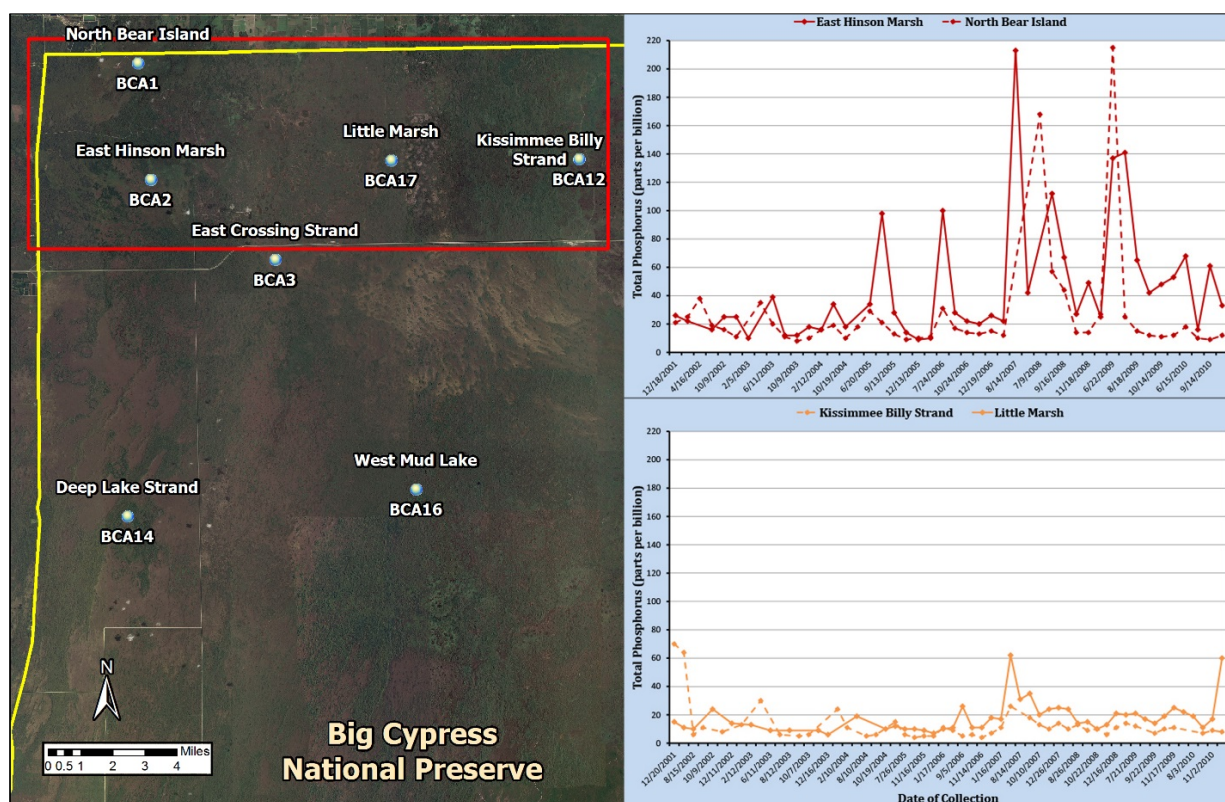


Figure B-6. Left, water-quality stations within the area of concern as indicated with a red border. Right, total phosphorus levels over a ten-year period, in parts-per-billion, for the four water-quality stations.

The time period the active or live diatom community in the periphyton sample collection represents has not been well studied, is not easily determined and depends on the substrate turnover rate. We provide some studies with relevant information below to provide an estimate. Theoretically, the floating mat collection should represent the last growing season, containing both the current live diatoms and the early wet season diatoms that have died but whose frustules are still encompassed in the floating mat. It has been found that when comparing periphyton communities collected from periphytometers (bare collection plates made of Plexiglas® which are collected serially) and nearby floating mat grab samples, that it takes about eight weeks for the two methods to begin to have similar diatom communities (Gaiser et al. 2006). Calcareous periphyton communities disappear quickly once nutrients have been added to the system. McCormick et al. (1996 and 2001) reported five-month-post TP enrichment loss of calcareous periphyton mat and conversion to filamentous green algae. Gaiser et al. (2006) reported loss of all calcareous periphyton mat in a long-term dosing project (even for the lowest dosing levels after four years of dosing). Taken together with the above findings this protocol assumes calcareous periphyton collections will represent the last wet season at a maximum and conservatively the two months prior to the collection.

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Appendix C. Justification for Basin Selection and Delineation

Urgelles, R. 2017. Justification for Basin Selection and Delineation. South Florida/Caribbean Network. Palmetto Bay, Florida.

The South Florida/Caribbean Network focused monitoring efforts in the northwest portion of the Big Cypress National Preserve (BICY). Geographical regions encompassing distinct hydrological basins were delineated following natural and artificial barriers, and designated a priori as impacted or unimpacted, based on water-quality data collected from nearby hydro stations. For the purpose of this monitoring, we use the term basin to describe these regions within the preserve. Water basins are either mainly rain-driven or have a canal inflow component to them and the flow characteristics considered are input source and direction of sheet flow. As both artificial barriers and the geology are semi-permeable, during periods of high water, different basins can become more hydrologically connected (e.g., water flows over trails). Basin delineation is approximate and based on basin information (Klein 1970) and locations of associated water-quality stations, both provided by the preserve's staff (R. Sobczak and P. Murphy, NPS, personal communication, 2008). Water flow directions are based on Klein (1970) and conversations with BICY staff.

Initially, six basins were delineated and designated a priori as “impacted” or “unimpacted,” based on total phosphorus (TP) data collected from the water-quality station within each basin's boundary (Figure C-1). A seventh and eighth basin were added, following the same criteria as the previous six. All eight basins are described in the sections and maps in this appendix. Two of these basins, Okaloacoochee Slough and East Hinson Marsh, are affected by canal inflow and are considered impacted as a result of upstream development or nutrient input from agricultural runoff. A third basin, Little Marsh, although not directly affected by canal inflow, demonstrated higher-than-usual water-column TP readings and was designated as unknown. The Bear Island basin is the only basin without an associated water-quality station. This region was sampled because it is located between the Okaloacoochee Slough, an impacted basin, and Little Marsh, an unknown basin. The other three basins have rain-driven sheet flow and are considered unimpacted: Fire Prairie, East Crossing Strand, and Monument.

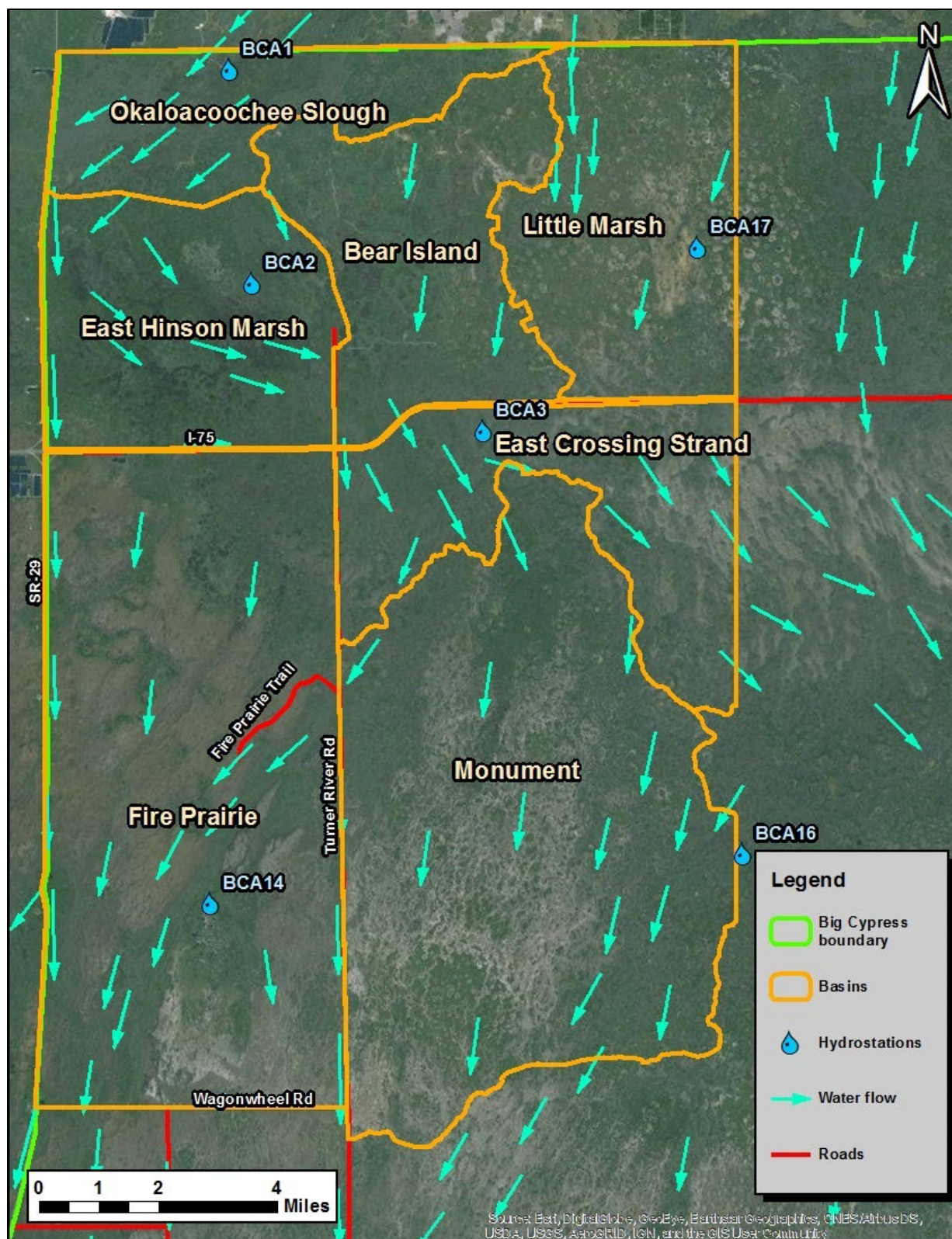


Figure C-1. Big Cypress National Preserve northwest corner with seven basins depicted (same legend used in the rest of the figures.). Cyan arrows represent hypothetical water flow directions are based on Klein (1970).

Okaloacoochee Slough

The Okaloacoochee Slough basin is located in the northwest corner of Big Cypress National Preserve and is bounded on the west by a road (SR 29) and on the south by ORV trails (Bear Island Grade, Hardrock Trail, Windmill Trail and Plains Trail). Flow moves in a southwesterly direction (Figure C-2). All the culverts are clustered along the Bear Island Grade and Hardrock Trail, and there appear to be none on the Windmill or Plains Trails. The lack of culverts in the eastern half of this basin, combined with the upland areas, creates different flow characteristics from those in the western half. Because there is no barrier to water flowing across this basin and there may be water discharge into the eastern section from just north of the preserve, this area is part of the Okaloacoochee basin. State Route 29 on the western boundary does not appear to allow much flow. This basin was designated as impacted based on high water-column TP. Okaloacoochee Slough basin appears to be affected by canal inflow.

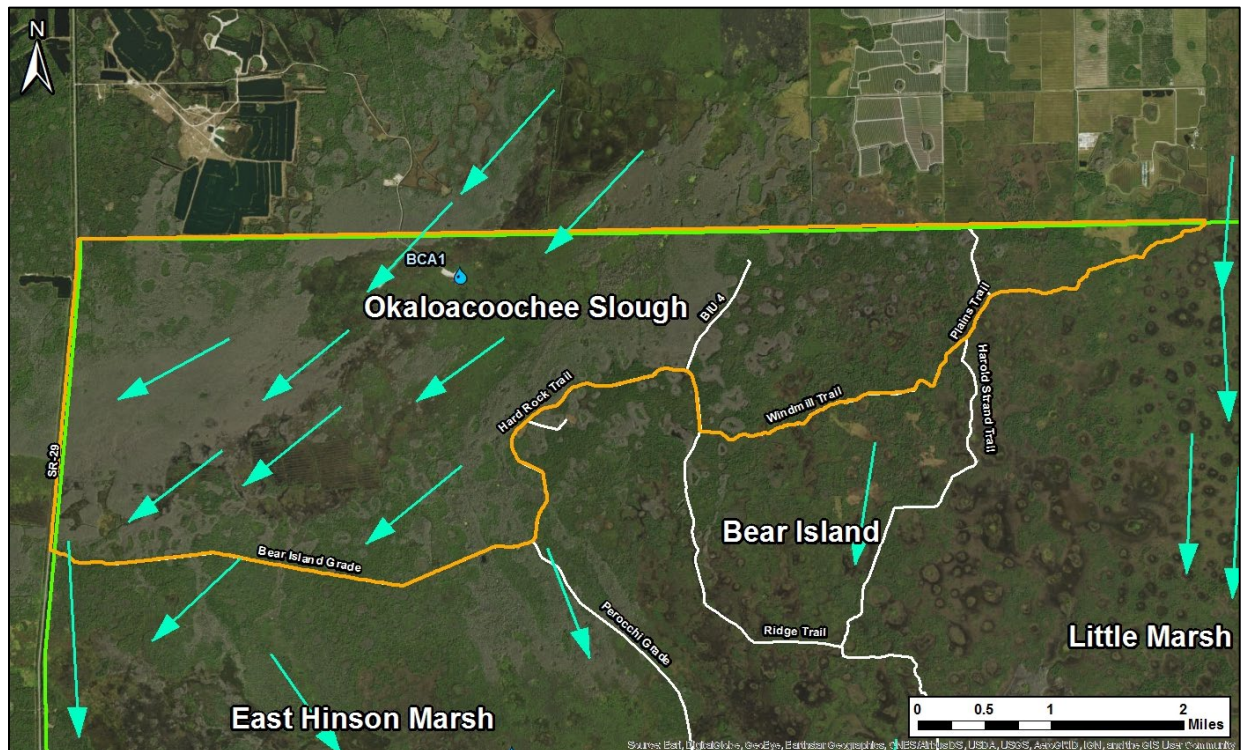


Figure C-2. Okaloacoochee Slough Basin.

East Hinson Marsh

The East Hinson Marsh basin is located immediately south of Okaloacoochee Slough. Interstate 75 is the southern boundary, SR 29 is the western boundary, Bear Island Grade ORV trail is the northern boundary and Perocchi Grade and Turner River Road are boundaries on the east. Flow input is from Okaloacoochee Slough and moves southwest, then southeast and then east (Figure C-3). This is possibly a result of the pinelands (and other woodlands) in the southwest corner of the basin, combined with the SR 29 barrier. This basin is designated as impacted because of high water-column TP. As with Okaloacoochee Slough, East Hinson Marsh appears to be affected by canal inflow.

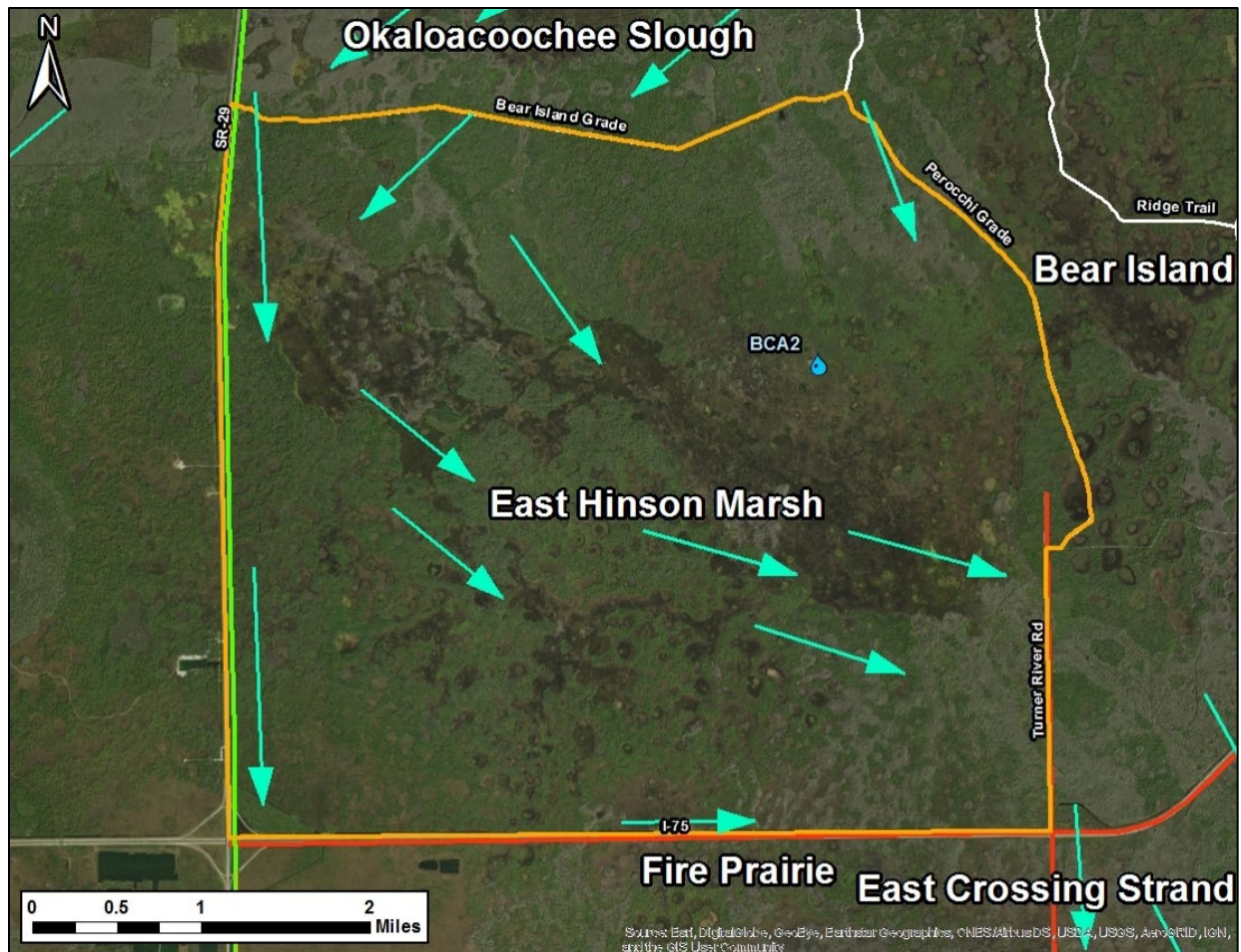


Figure C-3. East Hinson Marsh Basin.

Fire Prairie

The Fire Prairie basin encompasses the Deep Lake hydro-station BICYA14. Inputs are from rainfall. It is bounded on three sides by roads and appears to have two major flow patterns: one moving south from I-75 in the top half of the basin and the other moving southwest from Turner River Road (SR 839), just south of the Fire Prairie Trail (Figure C-4). This basin is designated as unimpacted based on low water-column TP.

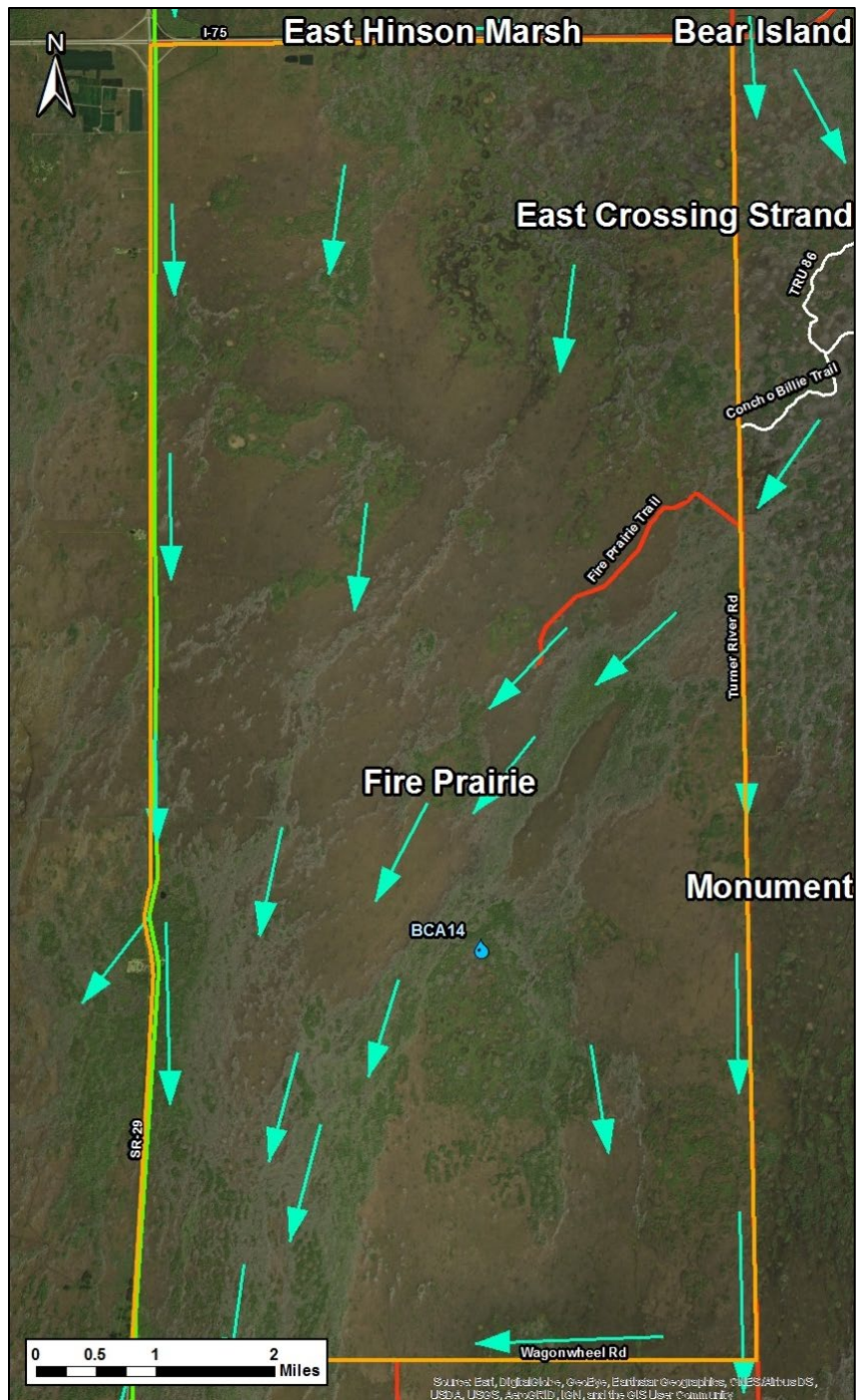


Figure C-4. Fire Prairie Basin.

Monument

The Monument basin was selected as one of the original unimpacted basins to be used for reference. The Monument basin has the water quality station BCA16. Inputs are from rainfall. It is bounded by Turner River Road on the west side and the Concho Billie trail to the north, east and south (Figure C-5).

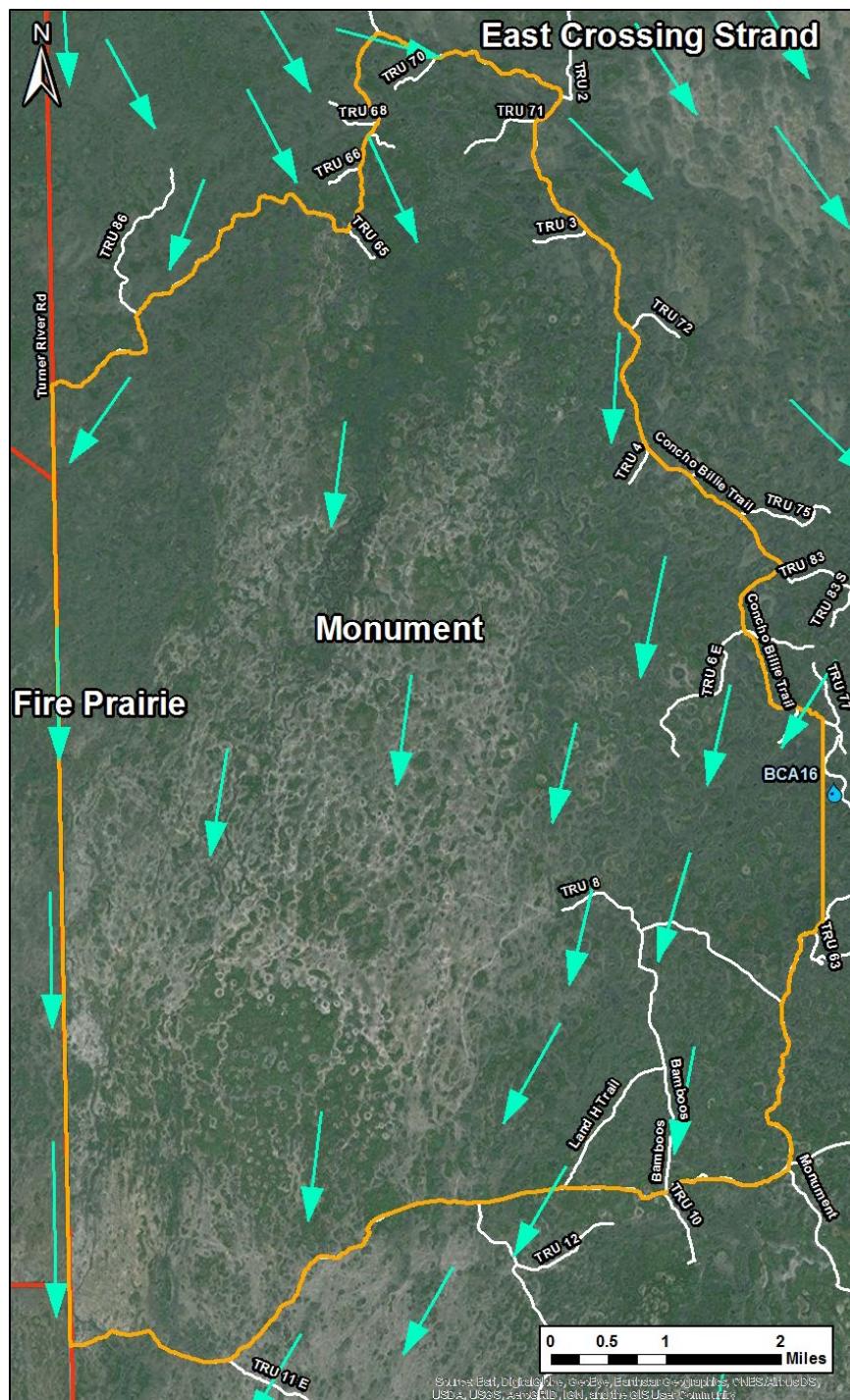


Figure C-5. Monument Basin.

East Crossing Strand

East Crossing Strand basin is bounded by I-75 on the north and the Concho Billie trail on the south. Flow originates from East Hinson Marsh and moves from underneath I-75 via culverts towards the south/southeast (Figure C-6). It appears that the Concho Billie trail does act as a barrier to flow. The

western half consists largely of cypress, while the eastern half opens up and appears to contain more marsh. This basin is designated as unimpacted based on low water-column TP.

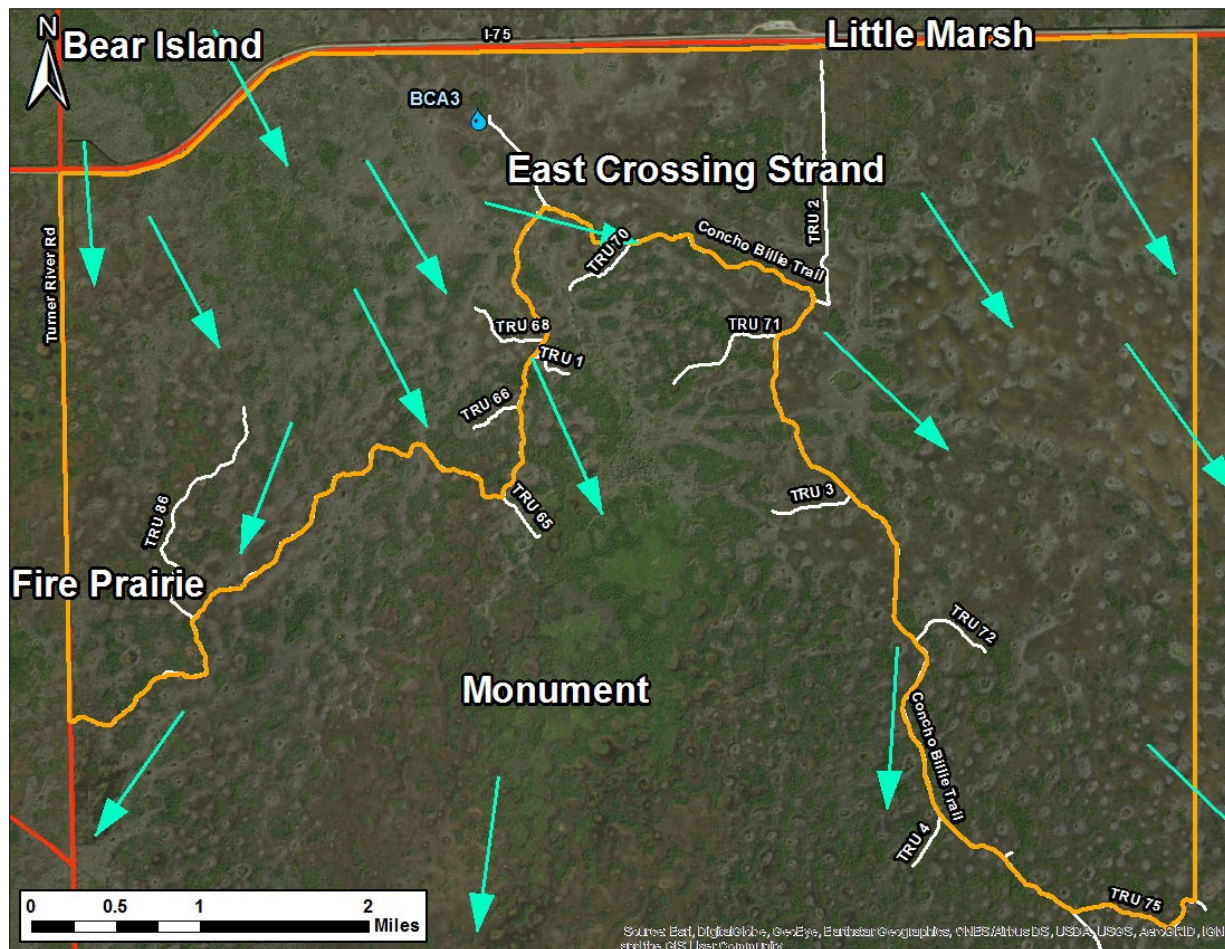


Figure C-6. East Crossing Strand Basin.

Little Marsh (also called Kissimmee Billy Strand in previous reports)

The Little Marsh basin encompasses hydro stations BICYA17 and BICYA12. Flow input is from outside Big Cypress National Preserve. On the south end of the basin is I-75. This basin is designated as unknown based on moderately high water-column TP (Figure C-7).

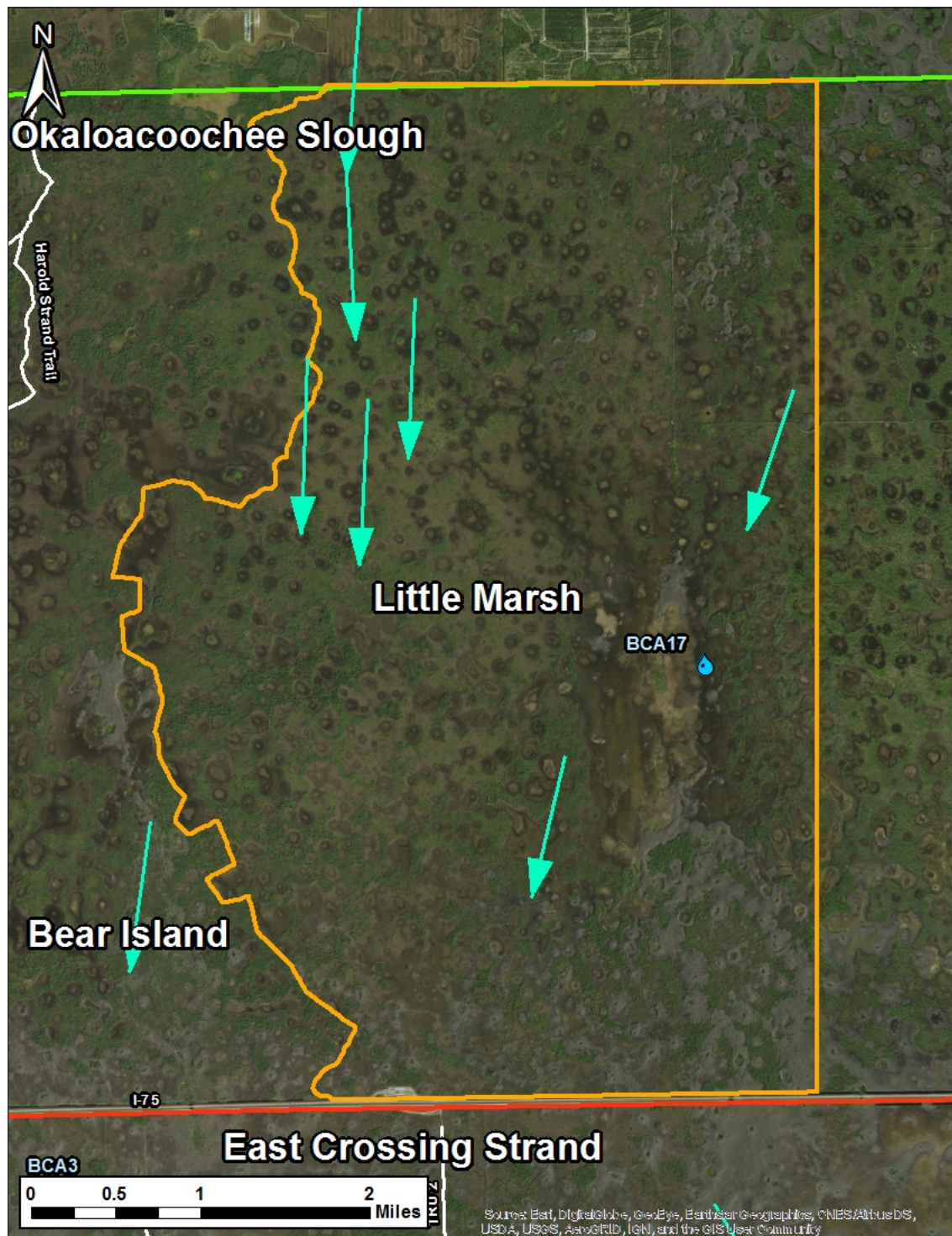


Figure C-7. Little Marsh (Kissimmee Billy Strand) Basin.

Kissimmee Billy (also called Kissimmee Billy East in previous reports)

Kissimmee Billy is located directly east of Little Marsh. Water-column TP levels exceeded normal background levels and thus this basin was also designated a priori as unknown. The area was

monitored from January 2012 to November 2013 but was not included in the current sampling design as it is outside of the Western Big Cypress National Preserve vegetation map footprint (Figure C-8).

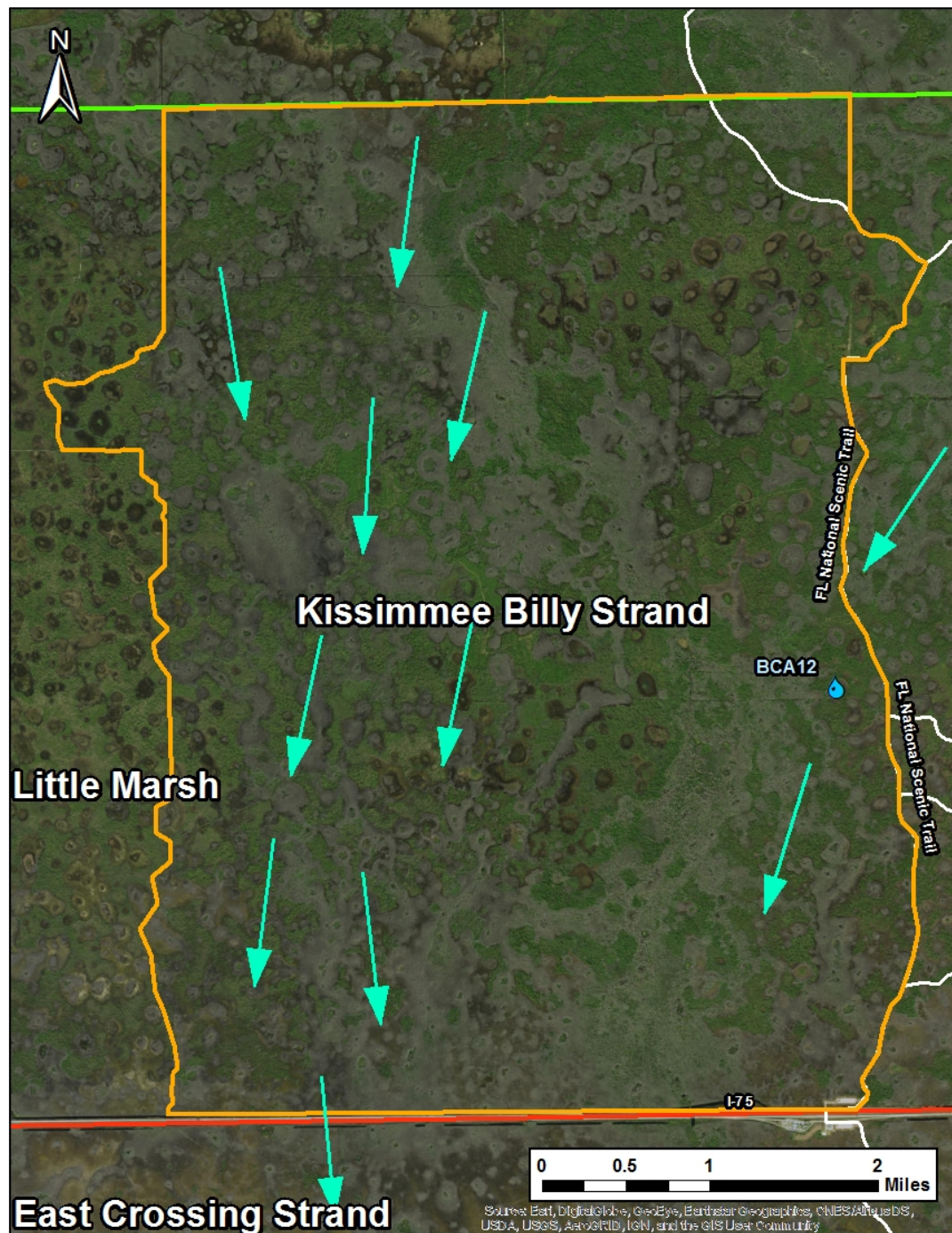


Figure C-8. Kissimmee Billy Basin.

Bear Island

The Bear Island basin is the only basin without an associated water-quality station. This region was sampled because it is located between the Okaloacoochee Slough, an impacted basin, and Little Marsh, an unknown basin (Figure C-9).

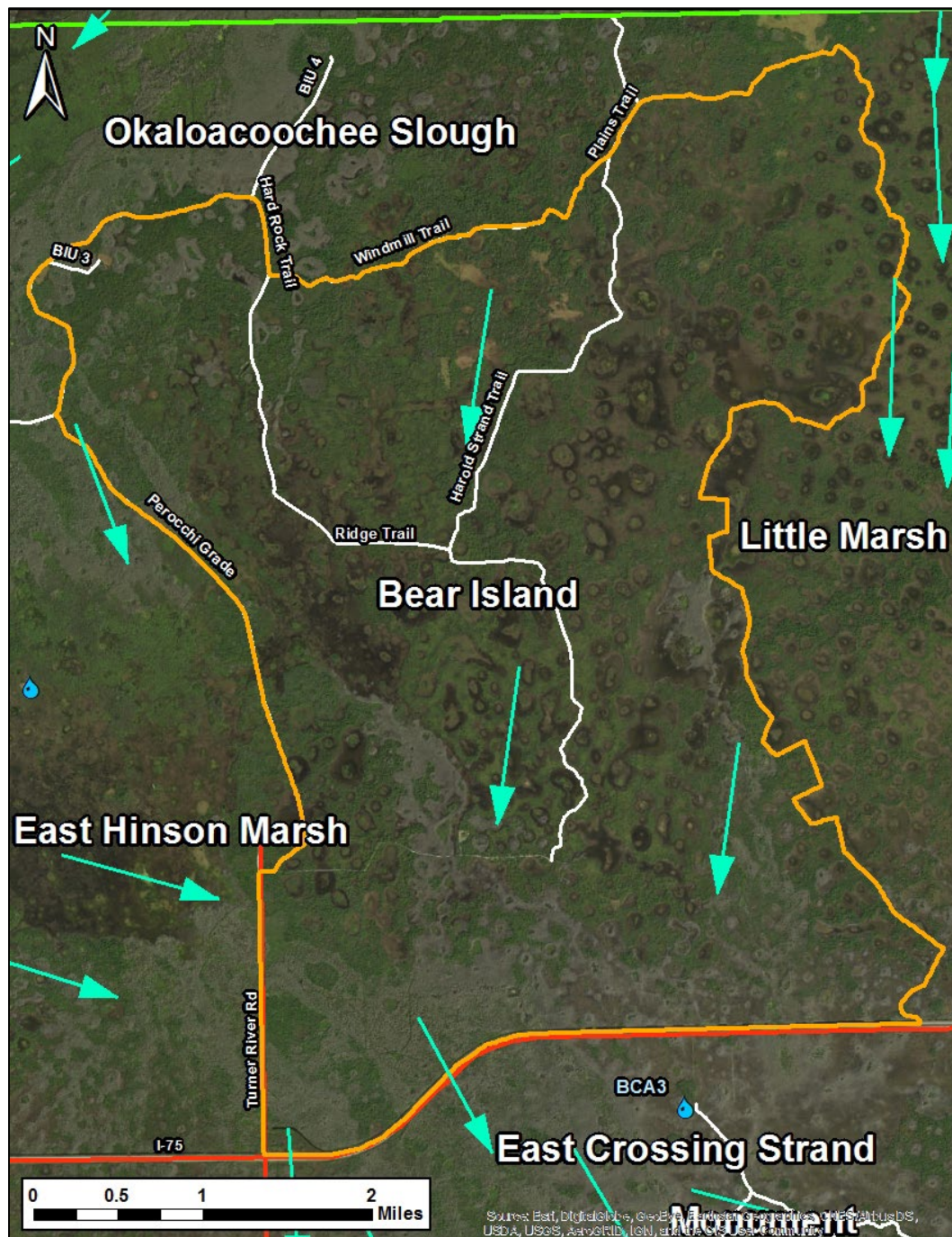


Figure C-9. Bear Island Basin.

Literature Cited

Klein, H., W. J. Schneider, B. F. McPherson, and T. J. Buchanan. 1970. Some hydrologic and biologic aspects of the Big Cypress Swamp drainage area. USGS Open-File Report 70003. 94 pp.

Appendix D. Database Tables and Definitions

Patterson, J. 2018. Database description and table definitions for periphyton collection in Big Cypress National Preserve. South Florida/Caribbean Network. Palmetto Bay, Florida.

The periphyton database (Figure D-1) is located in:

Z:\SFCN\Vital_Signs\Periphyton\data\SFCN_Periphyton.mdb.

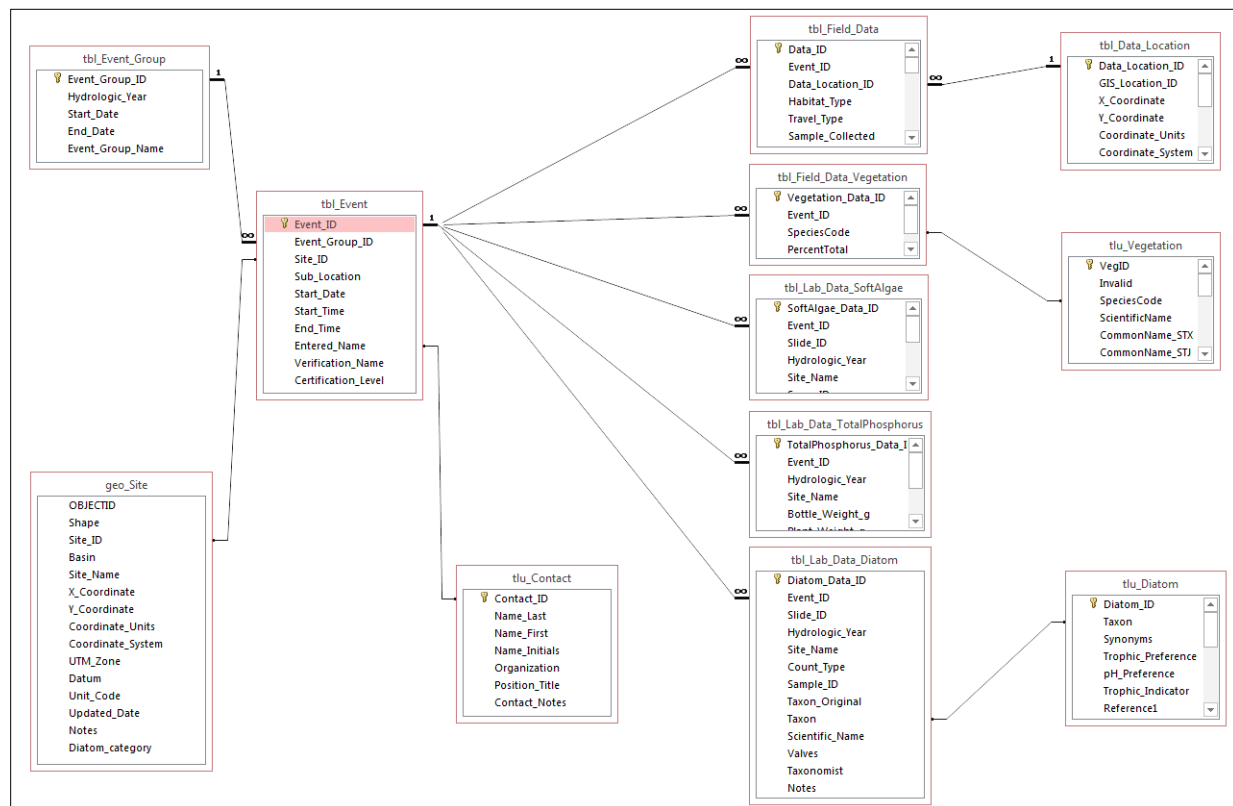


Figure D-1. Schema diagram showing the primary tables and relationships in the periphyton database.

The database was designed as a Microsoft Access geodatabase using the Natural Resource Database Template as a guide. The database stores four general types of data:

- **Field data**—these data are manually entered directly into the Microsoft Access database (SOP 9 *Data Entry and Quality Assurance* [Urgelles and Shamblin 2017]).
- **Spatial Data**—these geospatial feature classes include Permanent Monitoring Sites (geo_Site), Basins (geo_Basins), and Annual Sample Coordinates (geo_Sample_Location). The Annual Sample Coordinates shapefile holds new waypoints that are appended after each field season. This process is described in detail in SOP 8 *GPS Track and Waypoint Downloading and Archiving* (Shamblin 2017b). GPS tracklines are stored in shapefiles outside the geodatabase.

- Lab data—these data include processed data provided by a contracted lab. They represent the results of any soft algae, diatom, and/or nutrient analysis. These data need to be imported into the database and linked to the Field and Spatial Data (SOP 15 *Importing Laboratory Data* [Londoño and Patterson 2017]).
- Lookup lists—these lists are used to ensure consistency in data entry and reporting. They include information such as taxonomy, project staff, substrate types, and park units.

Core database tables and relationships are shown in Figure D-1. Tables D-1 through D-12 provide the definitions for each data field within each table.

Table D-1. tbl_Event_Group. This table stores event aggregations. Each row is a group that combines all of the sampling events for a single hydrologic year.

Field Name	Data Type	Description
Event_Group_ID	AutoNumber	A unique identifier
Hydrologic_Year	Number	Hydrologic year when the sampling took place; the hydrologic year starts on May 1, and runs until April 30th of the following year
Start_Date	Date/Time	Date of the first periphyton survey for a particular hydrologic year
End_Date	Date/Time	Date of the last periphyton survey for a particular hydrologic year
Event_Group_Name	Short Text	Text name/description for each periphyton event group

Table D-2. geo_Site. This geodatabase feature class stores the general sites/locations where monitoring occurs.

Field Name	Data Type	Description
OBJECTID	AutoNumber	Unique identifier (ESRI field)
Shape	OLE Object	Geometry for the site (ESRI field)
Site_ID	Number	Unique location identifier
Basin	Short Text	Name of the larger sub-watershed delineation separated by artificial or natural structures where the site is found
Site_Name	Short Text	Name of the site
X_Coordinate	Number	Easting (or X) coordinate
Y_Coordinate	Number	Northing (or Y) coordinate
Coordinate_Units	Short Text	Units of the coordinate system
Coordinate_System	Short Text	Name of the coordinate system

Table D-2 (continued). geo_Site. This geodatabase feature class stores the general sites/locations where monitoring occurs.

Field Name	Data Type	Description
UTM_Zone	Short Text	Universal Transverse Mercator zone number and letter
Datum	Short Text	Horizontal datum
Unit_Code	Short Text	4-letter park unit code
Updated_Date	Date/Time	Date this location was last updated
Notes	Short Text	Applicable comments

Table D-3. tbl_Event. This table stores visits to a location to collect a sample.

Field Name	Data Type	Description
Event_ID	AutoNumber	Unique identifier for the event.
Event_Group_ID	Number	Link to tbl_Event_Group
Site_ID	Number	Link to geo_Site
Sub_Location	Short Text	An optional identifier at a specific location (pilot work only)
Start_Date	Date/Time	Date that a location was visited
Start_Time	Date/Time	Starting time for a visit to a location
End_Time	Date/Time	Ending time for a visit to a location
Entered_Name	Number	Contact_ID of the person who did the data entry
Verification_Name	Number	Contact_ID of the person who did data verification
Certification_Level	Short Text	QA/QC processing level (as defined by QAP)

Table D-4. tbl_Field_Data. This table stores protocol-specific field data which is collected via datasheet.

Field Name	Data Type	Description
Data_ID	AutoNumber	Unique identifier
Event_ID	Number	Link to tbl_Events
Data_Location_ID	Number	Link to tbl_Data_Locations
Habitat_Type	Short Text	Dominant vegetation type at the sample location (Broadleaf marsh, Graminoid marsh, Cypress dome, Ecotone, or Other)

Table D-4 (continued). tbl_Field_Data. This table stores protocol-specific field data which is collected via datasheet.

Field Name	Data Type	Description
Travel_Type	Short Text	Method of transport used to reach the sampling location
Sample_Collected	Yes/No	Was a periphyton sample collected at this location?
GPS_Mark_Sample	Short Text	Waypoint identifier for the GPS coordinate where the field sample was taken
GPS_Mark_Landing	Short Text	Waypoint identifier for GPS coordinate at the helicopter landing spot
GPS_Unit	Short Text	Identifying name (on the back of the unit) of the GPS unit used
Photos	Number	Number of photographs associated with this sampling event
Observer_1	Number	Contact_ID of the first person involved in sampling
Observer_2	Number	Contact_ID of the second person involved in sampling
ORV_Activity	Short Text	Description of the vegetative damage from off-road vehicles that is visible near the sampling location (High, Medium, Low, or None)
Water_Present	Yes/No	Was the sample collected from standing water?
Water_Color	Short Text	The color of the water where sampling occurred (Clear, Slightly Amber, Amber)
Water_Depth1_cm	Number	First water depth (centimeters) reading at the sampling location
Water_Depth2_cm	Number	Second water depth (centimeters) reading at the sampling location
Water_Depth3_cm	Number	Third water depth (centimeters) reading at the sampling location
Water_Transparency_Depth_cm	Number	Depth (centimeters) at which the bottom of the ruler is no longer visible
Water_Temp_C	Number	Water temperature in degrees Celsius recorded with YSI meter at the sampling location
Water_Ambient_Conductivity	Number	Water ambient conductivity in μ S recorded with YSI meter at the sampling location
Substrate_Type	Short Text	Dominant covering of the substrate at the sample collection location
Substrate_Location	Short Text	Location of the substrate at the sample collection location (Floating, Ground, or Sweaters)
Coverage_Vegetation	Number	Absolute percent cover of vegetation in the 5 meter radius circle sample collection location
Coverage_Periphyton	Number	Absolute percent cover of periphyton in the 5 meter radius circle sample collection location

Table D-4 (continued). tbl_Field_Data. This table stores protocol-specific field data which is collected via datasheet.

Field Name	Data Type	Description
Coverage_OpenWater	Number	Absolute percent cover of open water in the 5 meter radius circle sample collection location
Notes	Short Text	Applicable comments

Table D-5. tbl_Field_Data_Vegetation. This table stores vegetation data collected during a sampling visit.

Field Name	Data Type	Description
Vegetation_Data_ID	AutoNumber	Unique identifier
Event_ID	Number	Link to tbl_Event
SpeciesCode	Short Text	Plant species code corresponding to first three letters of genus and first three letters of species
PercentTotal	Number	Absolute percent cover of a single SpeciesCode in the 5 meter radius circle sample collection location
Notes	Short Text	Applicable comments

Table D-6. tbl_Lab_Data_SoftAlgae. This table stores algae identification and count data from the lab.

Field Name	Data Type	Description
SoftAlgae_Data_ID	AutoNumber	Unique identifier
Event_ID	Number	Link to tbl_Events
Slide_ID	Short Text	Unique slide identification name assigned by the lab
Hydrologic_Year	Number	Hydrologic year when the sampling took place
Site_Name	Short Text	Name of the location where the sample was collected
Samp_ID	Short Text	Identification label placed on sample bottle
Count_Type	Short Text	Type of count that is stored in this table
Taxon_Original	Short Text	Scientific name as provided by contractor; sometimes includes intraspecific labels and authority
Scientific_Name	Short Text	Binomial scientific name stripped down to just the genus and species
Counts_Natural_Units	Number	Count of natural units (each individual filament, colony, or isolated cell is a natural unit)

Table D-6 (continued). tbl_Lab_Data_SoftAlgae. This table stores algae identification and count data from the lab.

Field Name	Data Type	Description
Counts_Cells	Number	Count of cells
Notes	Short Text	Applicable comments

Table D-7. tbl_Lab_Data_TotalPhosphorus. This table stores total phosphorus data from the lab.

Field Name	Data Type	Description
TotalPhosphorus_Data_ID	AutoNumber	Unique identifier
Event_ID	Number	Link to tbl_Events
Hydrologic_Year	Number	Hydrologic year when the sampling took place
Site_Name	Short Text	Name of the location where the sample was collected
Bottle_Weight_g	Number	Dry weight in grams of container
Plant_Weight_g	Number	Dry weight in grams of all associated macrophytes/substrates removed from the sample
Sample_Wet_Weight_g	Number	Actual periphyton wet weight in grams
Total_Phosphorus	Number	Total phosphorus ($\mu\text{g/g}$ of dried material)
Notes	Short Text	Applicable comments

Table D-8. tbl_Lab_Data_Diatom. This table stores diatom identification and count data from the lab.

Field Name	Data Type	Description
Diatom_Data_ID	AutoNumber	Unique identifier
Event_ID	Number	Link to tbl_Events
Slide_ID	Short Text	Slide identification name assigned by the lab
Hydrologic_Year	Number	Hydrologic year when the sampling took place
Site_Name	Short Text	Name of the site where the sample was collected
Sample_ID	Short Text	Identification label placed on sample bottle
Count_Type	Short Text	Type of count that is stored
Taxon_Original	Short Text	Scientific name as provided by contractor; sometimes includes intraspecific labels and authority

Table D-8 (continued). tbl_Lab_Data_Diatom. This table stores diatom identification and count data from the lab.

Field Name	Data Type	Description
Taxon	Short Text	Scientific name stripped down to include genus, species, form, and variety
Scientific_Name	Short Text	Binomial scientific name stripped down to just the genus and species
Valves	Number	Count of valves (one side of a diatom) of a specific taxon that are visible by microscope on a slide
Taxonomist	Short Text	Full name of the person who made the diatom identifications
Notes	Short Text	Applicable comments

Table D-9. tbl_Data_Location. This table stores the specific coordinates where samples are collected.

Field Name	Data Type	Description
Data_Location_ID	AutoNumber	Unique identifier
GIS_Location_ID	Short Text	Link to GIS feature
X_Coordinate	Number	Easting (or X) coordinate where the actual sample was collected
Y_Coordinate	Number	Northing (or Y) coordinate where the actual sample was collected
Coordinate_Units	Short Text	Units of the coordinate system
Coordinate_System	Short Text	Name of the coordinate system
UTM_Zone	Short Text	Universal Transverse Mercator zone number and letter
Datum	Short Text	Horizontal datum
Estimated_Horizontal_Error_m	Number	Estimated horizontal accuracy in meters
Accuracy_Notes	Short Text	Positional accuracy notes
Unit_Code	Short Text	4-letter park unit code
Updated_Date	Date/Time	Date of entry or last change
Notes	Short Text	General notes on the sampling location

Table D-10. tlu_Vegetation. This lookup table stores South Florida and Caribbean vegetation codes.

Field Name	Data Type	Description
VegID	AutoNumber	Unique identifier
InvalidCode	Yes/No	Is this an invalid (out of date) veg code?
SpeciesCode	Short Text	Plant species code corresponding to first three letters of genus and first three letters of species
ScientificName	Short Text	Binomial scientific name stripped down to just the genus and species
CommonName_FL	Short Text	Most commonly used name in Florida
ScientificName_ITIS	Short Text	Full Scientific Name with authorities from the ITIS database
Family	Short Text	Taxonomic family
Nativity_SouthFlorida	Short Text	Nativity status for the plant in South Florida
DateAdded	Date/Time	Date the entry was added to the lookup
Notes	Short Text	Applicable comments

Table D-11. tlu_Diatom. This lookup table holds a list of diatoms and other reference information.

Field Name	Data Type	Description
Diatom_ID	AutoNumber	Unique identifier
Taxon	Short Text	Scientific name stripped down to include genus, species, form, and variety
Synonyms	Short Text	Additional name(s) for this taxon
ScientificName_Full	Short Text	Full scientific name with authorities from USGS Biodata
USGS Biodata Version	Short Text	Version number for USGS Biodata from which full scientific name is drawn
Trophic_Preference	Short Text	Literature derived environmental nutrient preference
pH_Preference	Short Text	Literature derived environmental pH preference
Trophic_Indicator	Yes/No	Does the literature indicate that this diatom is a trophic indicator?
Reference1	Short Text	First reference used to verify diatom details
Reference2	Short Text	Second reference used to verify diatom details
Reference3	Short Text	Third reference used to verify diatom details
Reference4	Short Text	Fourth reference used to verify diatom details

Table D-11 (continued). tlu_Diatom. This lookup table holds a list of diatoms and other reference information.

Field Name	Data Type	Description
Mat_TP_Optima	Number	Abundance weighted average for total phosphorus in periphyton mats
Mat_TP_Tolerance	Number	Abundance weighted standard deviation for total phosphorus in periphyton mats
Soil_TP_Optima	Number	Abundance weighted average for total phosphorus in soil
Soil_TP_Tolerance	Number	Abundance weighted standard deviation for total phosphorus in soil
H2O_TP_Optima	Number	Abundance weighted average for total phosphorus in water
H2O_TP_Tolerance	Number	Abundance weighted standard deviation for total phosphorus in water
FullSpecies	Yes/No	Is this taxon at the species level?
Notes	Short Text	Applicable comments

Table D-12. tlu_Contact. This lookup table stores data for project-related personnel.

Field Name	Data Type	Description
Contact_ID	AutoNumber	Unique identifier
Name_Last	Short Text	Last name of the contact
Name_First	Short Text	First name of the contact
Organization	Short Text	Organization or employer of the contact
Position_Title	Short Text	Title or position description of the contact
Notes	Short Text	Applicable comments

Literature Cited

- Londoño, M., and J. M. Patterson. 2019. Standard operating procedure 9: Importing laboratory data. South Florida/Caribbean Network, Palmetto Bay, Florida.
- Shamblin, R. B. 2019. Standard operating procedure 8: GPS track and waypoint downloading and archiving. South Florida/Caribbean Network, Palmetto Bay, Florida.
- Urgelles, R., and R. B. Shamblin 2019. Standard operating procedure 9: Data entry and quality assurance. South Florida/Caribbean Network. National Park Service, Palmetto Bay, Florida.

Appendix E. Big Cypress National Preserve Work Forms

Shamblin, R. B. 2017. Big Cypress National Preserve work forms. South Florida/Caribbean Network. Palmetto Bay, Florida.

Certain forms need to be completed while working in Big Cypress National Preserve (BICY). The Aircraft Flight Request/Schedule form is sent out prior to field work when conducting work from a helicopter. The SFCN Periphyton Monitoring Site Evaluation form and the SFCN Periphyton Field Datasheet are forms filled out while at a site.

Aircraft Flight Request/Schedule Form (Figure E-1): To reserve an aircraft for field work an Aircraft Flight Request/Schedule Form must be filled out and submitted to Big Cypress National Preserve Fire and Aviation approximately 72 hours before the flight. Send a list of the site names and coordinates, and a map of the sites, along with the Aircraft Flight Request/Schedule Form.

SFCN Periphyton Monitoring Site Evaluation Form: The SFCN Periphyton Monitoring Site Evaluation Form applies to the evaluation of potential sampling sites. This task may need to be completed through both land and aerial observations. This evaluation will take place once, prior to sampling. Once the evaluation of the sites has taken place, the sites should not have to be re-evaluated unless conditions at the site change, affecting the landscape (possibly due to storm or long term vegetation growth). An example of the Initial Site Evaluation for Periphyton Sampling Sites form can be found in SOP 3 *Initial Site Evaluation for Periphyton Sampling Sites* (Figure 1; Shamblin 2019).

SFCN Periphyton Field Datasheet: The SFCN Periphyton Field Datasheet is filled out at the periphyton sampling site when periphyton sampling is taking place. These datasheets are generated in ArcMap and associate aerial imagery with the site ID. An example of a Field Datasheet can be found in SOP 1 *Field Mission Preparation: Creating Datasheets* (Figure 3; Urgelles 2019).

UNITED STATES DEPARTMENT OF THE INTERIOR AIRCRAFT FLIGHT REQUEST/SCHEDULE										Change #		6. Aircraft Information FAA N#											
1. Initial request information			Cost-Account/Management Code(s)				Billee Code (OAS A/C only)				Flight Schedule No.		PAX Seats										
Initial Date/Time	To/From EVER Flight Following/	Phone Numbers									Make/Model												
											Color												
Check one: ☐ Point-to-Point Flight ☒ Mission Flight												Desired A/C Type: ☒ Helicopter ☐ Airplane		Vendor									
Mission Objective/Special Needs:												Phone No.											
												Pilot(s)											
2. Passenger/Cargo Information – Indicate Chief of Party with an asterisk (*) Please include pax cell numbers																							
NAME/TYPE OF CARGO		LBS OR CU FT	PROJECT ORDER/ REQUEST NO.		DEPT ARPT	DEST ARPT	RETURN TO	NAME/TYPE OF CARGO		LBS OR CU FT	PROJECT ORDER/ REQUEST NO.		DEPT ARPT	DEST ARPT	RETURN TO								
3. Flight Itinerary (For Mission-Type Flights, Provide Points of Departure/Arrival and Attach Map with Detailed Flight Route and Known Hazards Indicated)																							
DEPART WITH			DEPART FROM			ENROUTE	ARRIVE AT			DROP OFF		KEY POINTS				INFO RELAYED							
Date	No. Pax	Lbs.	Airport/Place		ETD	ATD	ETE	Airport/Place		ETA	ATA	No. Pax	Lbs.	Drop-Off Points, Refueling Stops, Flight Check-Ins, Pickup Points		To/From							
4. Flight Following																5. Method of Resource Tracking:				7. Administrative		8. Review (If applicable)	
☐ FAA IFR ☐ Satellite																☐ Phone ☒ Radio				Type of Payment Document:		☐ Hazard Analysis Performed	
☐ FAA VFR With Check-In Every _____ Minutes To																☐ To Scheduling Dispatcher @ _____ (Phone Number)				☒ OAS-23 or ☐ OAS 2		☐ Dispatch/Aviation Mgr. Checklist	
☐ FAA or ☐ Agency																☐ Prior to Takcoff ☐ Each Stop Enroute ☐ Arrival at Destination				☒ FFS 6500-122			
☒ Agency VFR With Check-In via radio Every <u>15</u> Minutes																☐ To: _____ @ _____ (Other Office) (Phone Number)				☐ Other		☐ Other:	
Frequency(ies):																				Route Document To:			
																				9. Close-out		Closed by: _____ Date/Time: _____	

Literature Cited

Shamblin, R. B. 2019. Initial Site Evaluation for Periphyton Sampling Sites—Version 1.0. South Florida/Caribbean Network Standard Operating Procedure. South Florida/Caribbean Network, Palmetto Bay, Florida.

Urgelles, R. 2019. Field Mission Preparation: Creating Datasheets—Version 1.0. South Florida/Caribbean Network Standard Operating Procedure. National Park Service, Palmetto Bay, Florida.

Appendix F. Diatom Species List and Indicator Status

Urgelles, R. 2017. Diatom Species List and Indicator Status in Big Cypress National Preserve. South Florida/Caribbean Network. Palmetto Bay, Florida.

The following table lists the diatoms observed since 2009 in Big Cypress National Preserve during periphyton collection performed by the South Florida Caribbean Network. Table F-1 shows the taxon, the abiotic features such as trophic preference and pH preference of the diatoms, as well the ability to use the taxon as an indicator species. References follow the table.

Table F-1. Diatom taxon, alternate names, reference(s), preference for Oligotrophic, Mesotrophic, or Eutrophic conditions, pH preference, and optimum soil, periphyton mat, and water total phosphorus (TP) and tolerance (tol). Taxonomic names from Jan Stevenson, Michigan State University (subject matter expert). The standard approach to obtain the weighted average optimum is to calculate the average of all the values of the particular environmental variable in which a particular species occurs, weighted by the species' relative abundance. This provides the weighted average optimum. The tolerance is the abundance-weighted standard deviation.

Taxon	Synonyms	Reference(s)	Trophic Preference	pH Preference	Trophic Indicator?	Soil TP Optima	Soil TP Tol	Mat TP Optima	Mat TP Tol	H ₂ O TP Optima	H ₂ O TP Tol
<i>Achnanthes brevipes</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Achnanthes coarctata</i>	–	4	Oligotrophic – Mesotrophic	Circumneutral	–	–	–	–	–	–	–
<i>Achnanthes minutissima</i> var. <i>jackii</i>	<i>Achnantheidium affine</i>	11, 4	Oligotrophic	Circumneutral	–	–	–	–	–	–	–
<i>Achnantheidium caledonicum</i>	<i>Achnanthes minutissima</i> var. <i>scotica</i> <i>Achnanthes caledonica</i>	6, 10, 12, 4	Oligotrophic	Circumneutral	Yes	444	82	277	221	31	13
<i>Achnantheidium exiguum</i>	<i>Achnanthes exigua</i>	6, 11, 12, 4	Eutrophic	Alkaliphilic	Yes	1,340	–	–	–	65	24
<i>Achnantheidium exilis</i>	<i>Achnanthes exilis</i>	4	Oligotrophic – Mesotrophic	Alkaliphilic	–	–	–	–	–	–	–
<i>Achnantheidium macrocephalum</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Achnantheidium minutissimum</i>	<i>Achnanthes minutissima</i>	11, 2, 9, 4	Oligotrophic – Mesotrophic	Circumneutral	–	–	–	–	–	35	–
<i>Achnantheidium</i> spp.	–	–	–	–	–	–	–	–	–	–	–
<i>Adlafia bryophila</i>	<i>Navicula bryophila</i>	11, 4	Mesotrophic – Eutrophic	Circumneutral	–	–	–	–	–	–	–

Table F-1 (continued). Diatom taxon, alternate names, reference(s), preference for Oligotrophic, Mesotrophic, or Eutrophic conditions, pH preference, and optimum soil, periphyton mat, and water total phosphorus (TP) and tolerance (tol). Taxonomic names from Jan Stevenson, Michigan State University (subject matter expert). The standard approach to obtain the weighted average optimum is to calculate the average of all the values of the particular environmental variable in which a particular species occurs, weighted by the species' relative abundance. This provides the weighted average optimum. The tolerance is the abundance-weighted standard deviation.

Taxon	Synonyms	Reference(s)	Trophic Preference	pH Preference	Trophic Indicator?	Soil TP Optima	Soil TP Tol	Mat TP Optima	Mat TP Tol	H ₂ O TP Optima	H ₂ O TP Tol
<i>Adafia minuscula</i>	<i>Navicula minuscula</i>	6, 11, 4, 13	Oligotrophic – Eutrophic	Alkaliphilic	–	1,289	–	–	–	–	–
<i>Amphora copulata</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Amphora holsatica</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Amphora ovalis</i>	–	2	Mesotrophic - Eutrophic	Alkaliphilic	–	–	–	–	–	–	–
<i>Amphora pediculus</i>	–	9	Mesotrophic	–	–	–	–	–	–	47	–
<i>Amphora</i> spp.	–	–	–	–	–	–	–	–	–	–	–
<i>Amphora veneta</i>	–	6, 10, 12	Eutrophic	Alkaliphilic	Yes	1,332	191	1,730	625	–	–
<i>Anomoeoneis sphaerophora</i> f. <i>costata</i>	–	6	Eutrophic	–	–	1,036	–	–	–	–	–
<i>Asterionella ralfsii</i>	–	2	Mesotrophic	Acidophilic	–	–	–	–	–	–	–
<i>Aulacoseira ambigua</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Aulacoseira italica</i> f. <i>crenulata</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Aulacoseira granulata</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Aulacoseira herzogii</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Aulacoseira italica</i>	–	–	–	–	–	–	–	–	–	–	–

Table F-1 (continued). Diatom taxon, alternate names, reference(s), preference for Oligotrophic, Mesotrophic, or Eutrophic conditions, pH preference, and optimum soil, periphyton mat, and water total phosphorus (TP) and tolerance (tol). Taxonomic names from Jan Stevenson, Michigan State University (subject matter expert). The standard approach to obtain the weighted average optimum is to calculate the average of all the values of the particular environmental variable in which a particular species occurs, weighted by the species' relative abundance. This provides the weighted average optimum. The tolerance is the abundance-weighted standard deviation.

Taxon	Synonyms	Reference(s)	Trophic Preference	pH Preference	Trophic Indicator?	Soil TP Optima	Soil TP Tol	Mat TP Optima	Mat TP Tol	H ₂ O TP Optima	H ₂ O TP Tol
<i>Bacillaria paradoxa</i>	–	11	Eutrophic	–	–	–	–	–	–	–	–
<i>Brachysira exilis</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Brachysira microcephala</i>	<i>Brachysira neoexilis</i>	10, 11, 12	Oligotrophic	–	Yes	–	–	100	56	32	20
<i>Caloneis alpestris</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Caloneis bacillum</i>	–	6, 8, 12, 4	Eutrophic	Alkaliphilic	Yes	1,252	–	–	–	53	15
<i>Caloneis</i> spp.	–	–	–	–	–	–	–	–	–	–	–
<i>Caloneis sublinearis</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Chamaepinnularia evanida</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Chamaepinnularia soehrensii</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Cocconeis placentula</i> var. <i>lineata</i>	–	6, 8, 12, 4	Eutrophic	Alkaliphilic	Yes	1,228	–	–	–	41	22
<i>Cocconeis scutellum</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Craticula accomoda</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Craticula citrus</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Craticula cuspidata</i>	<i>Navicula cuspidata</i>	11, 8, 4	Eutrophic	Alkaliphilic	Yes	–	–	–	–	–	–

Table F-1 (continued). Diatom taxon, alternate names, reference(s), preference for Oligotrophic, Mesotrophic, or Eutrophic conditions, pH preference, and optimum soil, periphyton mat, and water total phosphorus (TP) and tolerance (tol). Taxonomic names from Jan Stevenson, Michigan State University (subject matter expert). The standard approach to obtain the weighted average optimum is to calculate the average of all the values of the particular environmental variable in which a particular species occurs, weighted by the species' relative abundance. This provides the weighted average optimum. The tolerance is the abundance-weighted standard deviation.

Taxon	Synonyms	Reference(s)	Trophic Preference	pH Preference	Trophic Indicator?	Soil TP Optima	Soil TP Tol	Mat TP Optima	Mat TP Tol	H ₂ O TP Optima	H ₂ O TP Tol
<i>Craticula halophilioides</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Craticula molestiformis</i>	<i>Navicula molestiformis</i>	11, 12, 4	Eutrophic	Alkaliphilic	Yes	–	–	–	–	74	3
<i>Cyclotella meneghiniana</i>	–	6, 3, 8, 2	Eutrophic	Alkaliphilic	Yes	1,251	–	313	325	–	–
<i>Cymbella aspera</i>	–	4	Oligotrophic - Eutrophic	Alkaliphilic	–	–	–	–	–	–	–
<i>Cymbella delicatula</i>	–	4, 13	Oligotrophic	Alkaliphilic	Yes	–	–	–	–	–	–
<i>Cymbella gracilis</i>	–	4	Oligotrophic - Mesotrophic	Acidophilic	–	–	–	–	–	–	–
<i>Cymbella mesiana</i>	<i>Encyonema mesianum</i>	6, 4, 13	Oligotrophic - Mesotrophic	Alkaliphilic	–	755	–	–	–	–	–
<i>Cymbella proxima</i>	–	4	Mesotrophic	–	–	–	–	–	–	–	–
<i>Denticula elegans</i>	–	2	–	Alkaliphilic	–	–	–	–	–	–	–
<i>Denticula kuetzingii</i>	–	6	Oligotrophic	–	–	478	–	–	–	–	–
<i>Denticula kuetzingii</i> var. <i>rumrichae</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Diadsmis confervacea</i>	<i>Navicula confervacea</i>	6, 11, 12, 1	Eutrophic	–	Yes	1,300	154	–	–	58	25

Table F-1 (continued). Diatom taxon, alternate names, reference(s), preference for Oligotrophic, Mesotrophic, or Eutrophic conditions, pH preference, and optimum soil, periphyton mat, and water total phosphorus (TP) and tolerance (tol). Taxonomic names from Jan Stevenson, Michigan State University (subject matter expert). The standard approach to obtain the weighted average optimum is to calculate the average of all the values of the particular environmental variable in which a particular species occurs, weighted by the species' relative abundance. This provides the weighted average optimum. The tolerance is the abundance-weighted standard deviation.

Taxon	Synonyms	Reference(s)	Trophic Preference	pH Preference	Trophic Indicator?	Soil TP Optima	Soil TP Tol	Mat TP Optima	Mat TP Tol	H ₂ O TP Optima	H ₂ O TP Tol
<i>Diadesmis contenta</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Diploneis boldtiana</i>	–	13	Oligotrophic	–	Yes	–	–	–	–	–	–
<i>Diploneis elliptica</i>	–	6	Mesotrophic	Alkaliphilic - Circumneutral	–	934	–	–	–	–	–
<i>Diploneis oblongella</i>	–	10, 11, 12, 4	Oligotrophic	Alkaliphilic	Yes	–	–	175	151	27	9
<i>Diploneis oculata</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Diploneis ovalis</i>	–	12	Mesotrophic	–	–	–	–	–	–	40	19
<i>Diploneis parma</i>	–	10	Mesotrophic	–	–	–	–	507	665	–	–
<i>Diploneis smithii</i>	–	6	Mesotrophic	–	–	937	–	–	–	–	–
<i>Diploneis subovalis</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Discostella stelligera</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Encyonema auerswaldii</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Encyonema gaeumanii</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Encyonema minutum</i>	–	11, 13	Oligotrophic	–	Yes	–	–	–	–	–	–
<i>Encyonema neomesianum</i>	–	12	Oligotrophic	–	–	–	–	–	–	26	13

Table F-1 (continued). Diatom taxon, alternate names, reference(s), preference for Oligotrophic, Mesotrophic, or Eutrophic conditions, pH preference, and optimum soil, periphyton mat, and water total phosphorus (TP) and tolerance (tol). Taxonomic names from Jan Stevenson, Michigan State University (subject matter expert). The standard approach to obtain the weighted average optimum is to calculate the average of all the values of the particular environmental variable in which a particular species occurs, weighted by the species' relative abundance. This provides the weighted average optimum. The tolerance is the abundance-weighted standard deviation.

Taxon	Synonyms	Reference(s)	Trophic Preference	pH Preference	Trophic Indicator?	Soil TP Optima	Soil TP Tol	Mat TP Optima	Mat TP Tol	H ₂ O TP Optima	H ₂ O TP Tol
<i>Encyonema silesiacum</i>	–	10, 11	Oligotrophic	–	–	–	–	195	90	–	–
<i>Encyonopsis cesatii</i>	–	13	Oligotrophic	–	–	–	–	–	–	–	–
<i>Encyonopsis evergladianum</i>	<i>Encyonema evergladianum</i>	6	Oligotrophic	–	Yes	468	138	169	116	26	16
<i>Encyonopsis falaisensis</i>	–	13	Oligotrophic	–	Yes	–	–	–	–	–	–
<i>Encyonopsis microcephala</i>	<i>Cymbella microcephala</i>	6, 10, 12, 13	Oligotrophic - Mesotrophic	–	–	747	–	266	194	33	11
<i>Encyonopsis vandamii</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Eolimna minima</i>	<i>Navicula minima</i>	–	–	–	–	–	–	–	–	–	–
<i>Eolimna subminuscula</i>	<i>Craticula subminuscula</i> <i>Navicula subminuscula</i>	–	–	–	–	–	–	–	–	–	–
<i>Epithemia adnata</i>	–	5, 8, 12	Eutrophic	Alkaliphilic	Yes	–	–	–	–	43	13
<i>Epithemia sorex</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Epithemia</i> spp.	–	–	–	–	–	–	–	–	–	–	–

Table F-1 (continued). Diatom taxon, alternate names, reference(s), preference for Oligotrophic, Mesotrophic, or Eutrophic conditions, pH preference, and optimum soil, periphyton mat, and water total phosphorus (TP) and tolerance (tol). Taxonomic names from Jan Stevenson, Michigan State University (subject matter expert). The standard approach to obtain the weighted average optimum is to calculate the average of all the values of the particular environmental variable in which a particular species occurs, weighted by the species' relative abundance. This provides the weighted average optimum. The tolerance is the abundance-weighted standard deviation.

Taxon	Synonyms	Reference(s)	Trophic Preference	pH Preference	Trophic Indicator?	Soil TP Optima	Soil TP Tol	Mat TP Optima	Mat TP Tol	H ₂ O TP Optima	H ₂ O TP Tol
<i>Eunotia bilunaris</i>	–	6, 11, 13	Mesotrophic - Eutrophic	–	–	838	–	–	–	–	–
<i>Eunotia camelus</i>	–	6	Eutrophic	–	–	1,545	–	–	–	–	–
<i>Eunotia flexuosa</i>	–	10, 3, 12, 13	Mesotrophic - Eutrophic	Acidophilic	–	–	–	344	249	40	15
<i>Eunotia formica</i>	–	8, 2,	Eutrophic	Acidophilic - Circumneutral	Yes	–	–	–	–	–	–
<i>Eunotia glacialis</i>	–	6, 2	Eutrophic	Acidophilic - Circumneutral	–	1,114	–	–	–	–	–
<i>Eunotia implicata</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Eunotia incisa</i>	–	10, 2	Eutrophic	Acidophilic	–	–	–	679	387	–	–
<i>Eunotia minor</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Eunotia monodon</i>	–	10, 11, 2	Oligotrophic - Mesotrophic	Acidophilic	–	–	–	614	301	–	–
<i>Eunotia naegeli</i>	–	6, 10, 2, 13	Mesotrophic - Eutrophic	Acidophilic	–	1,420	–	469	325	–	–
<i>Eunotia nymanniana</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Eunotia parallela</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Eunotia pectinalis</i>	–	11, 2	Oligotrophic	Circumneutral	–	–	–	–	–	–	–

Table F-1 (continued). Diatom taxon, alternate names, reference(s), preference for Oligotrophic, Mesotrophic, or Eutrophic conditions, pH preference, and optimum soil, periphyton mat, and water total phosphorus (TP) and tolerance (tol). Taxonomic names from Jan Stevenson, Michigan State University (subject matter expert). The standard approach to obtain the weighted average optimum is to calculate the average of all the values of the particular environmental variable in which a particular species occurs, weighted by the species' relative abundance. This provides the weighted average optimum. The tolerance is the abundance-weighted standard deviation.

Taxon	Synonyms	Reference(s)	Trophic Preference	pH Preference	Trophic Indicator?	Soil TP Optima	Soil TP Tol	Mat TP Optima	Mat TP Tol	H ₂ O TP Optima	H ₂ O TP Tol
<i>Eunotia pectinalis</i> var. <i>undulata</i>	–	2, 13	Eutrophic	Acidophilic - Circumneutral	Yes	–	–	–	–	–	–
<i>Eunotia praerupta</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Eunotia praerupta</i> var. <i>bigibba</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Eunotia rhynchocephala</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Eunotia soleirolii</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Eunotia</i> spp.	–	–	–	–	–	–	–	–	–	–	–
<i>Fragilaria capucina</i> var. <i>gracilis</i>	–	6, 12	Mesotrophic	–	–	961	–	–	–	40	30
<i>Fragilaria capucina</i> var. <i>mesolepta</i>	–	6	Mesotrophic	–	–	961	–	–	–	–	–
<i>Fragilaria crotonensis</i>	–	11, 2	Oligotrophic - Eutrophic	–	–	–	–	–	–	–	–
<i>Fragilaria famelica</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Fragilaria sepes</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Fragilaria</i> spp.	–	–	–	–	–	–	–	–	–	–	–

Table F-1 (continued). Diatom taxon, alternate names, reference(s), preference for Oligotrophic, Mesotrophic, or Eutrophic conditions, pH preference, and optimum soil, periphyton mat, and water total phosphorus (TP) and tolerance (tol). Taxonomic names from Jan Stevenson, Michigan State University (subject matter expert). The standard approach to obtain the weighted average optimum is to calculate the average of all the values of the particular environmental variable in which a particular species occurs, weighted by the species' relative abundance. This provides the weighted average optimum. The tolerance is the abundance-weighted standard deviation.

Taxon	Synonyms	Reference(s)	Trophic Preference	pH Preference	Trophic Indicator?	Soil TP Optima	Soil TP Tol	Mat TP Optima	Mat TP Tol	H ₂ O TP Optima	H ₂ O TP Tol
<i>Fragilaria synegrotesca</i>	–	6, 10, 12	Oligotrophic	–	Yes	542	252	270	202	22	18
<i>Fragilaria tenera</i>	–	11, 12	Oligotrophic	–	–	–	–	–	–	14	13
<i>Fragilaria vaucheriae</i>	–	2, 13	Eutrophic	Alkaliphilic	–	–	–	–	–	–	–
<i>Fragilaria vaucheriae</i> var. <i>capitellata</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Fragilariforma</i> spp.	–	–	–	–	–	–	–	–	–	–	–
<i>Gomphonema acuminatum</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Gomphonema affine</i>	–	10, 12	Oligotrophic - Mesotrophic	–	–	–	–	247	207	43	16
<i>Gomphonema angustatum</i>	–	11	Eutrophic	–	–	–	–	–	–	–	–
<i>Gomphonema angustum</i>	–	13	Eutrophic	–	Yes	–	–	–	–	–	–
<i>Gomphonema augur</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Gomphonema auritum</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Gomphonema clavatum</i>	–	6, 10	Mesotrophic	–	–	690	–	370	401	–	–

Table F-1 (continued). Diatom taxon, alternate names, reference(s), preference for Oligotrophic, Mesotrophic, or Eutrophic conditions, pH preference, and optimum soil, periphyton mat, and water total phosphorus (TP) and tolerance (tol). Taxonomic names from Jan Stevenson, Michigan State University (subject matter expert). The standard approach to obtain the weighted average optimum is to calculate the average of all the values of the particular environmental variable in which a particular species occurs, weighted by the species' relative abundance. This provides the weighted average optimum. The tolerance is the abundance-weighted standard deviation.

Taxon	Synonyms	Reference(s)	Trophic Preference	pH Preference	Trophic Indicator?	Soil TP Optima	Soil TP Tol	Mat TP Optima	Mat TP Tol	H ₂ O TP Optima	H ₂ O TP Tol
<i>Gomphonema contraturris</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Gomphonema gracile</i>	–	6, 7, 12	Mesotrophic	Alkaliphilic - Circumneutral	–	974	341	–	–	39	16
<i>Gomphonema insigne</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Gomphonema intricatum</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Gomphonema mexicanum</i>	–	11	Oligotrophic	–	–	–	–	–	–	–	–
<i>Gomphonema micropus</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Gomphonema minutum</i>	–	4, 13	Eutrophic	Circumneutral	Yes	–	–	–	–	–	–
<i>Gomphonema neonasutum</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Gomphonema olivaceoides</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Gomphonema parvulum</i>	–	6, 10, 12, 13	Eutrophic	–	Yes	1,234	302	3,304	2,984	52	20

Table F-1 (continued). Diatom taxon, alternate names, reference(s), preference for Oligotrophic, Mesotrophic, or Eutrophic conditions, pH preference, and optimum soil, periphyton mat, and water total phosphorus (TP) and tolerance (tol). Taxonomic names from Jan Stevenson, Michigan State University (subject matter expert). The standard approach to obtain the weighted average optimum is to calculate the average of all the values of the particular environmental variable in which a particular species occurs, weighted by the species' relative abundance. This provides the weighted average optimum. The tolerance is the abundance-weighted standard deviation.

Taxon	Synonyms	Reference(s)	Trophic Preference	pH Preference	Trophic Indicator?	Soil TP Optima	Soil TP Tol	Mat TP Optima	Mat TP Tol	H ₂ O TP Optima	H ₂ O TP Tol
<i>Gomphonema parvulum</i> var. <i>lagenula</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Gomphonema pumilum</i>	–	11	Oligotrophic	–	–	–	–	–	–	–	–
<i>Gomphonema sarcophagus</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Gomphonema septum</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Gomphonema</i> spp.	–	–	–	–	–	–	–	–	–	–	–
<i>Gomphonema tenellum</i>	–	12	Eutrophic	–	–	–	–	–	–	55	19
<i>Gomphonema truncatum</i>	–	11	Oligotrophic	–	–	–	–	–	–	–	–
<i>Gomphonema vibrioides</i>	–	12	Oligotrophic	–	–	–	–	–	–	28	16
<i>Gomphosphenia grovei</i>	–	11	Oligotrophic	–	–	–	–	–	–	–	–
<i>Gyrosigma acuminatum</i>	–	11	Eutrophic	–	–	–	–	–	–	–	–
<i>Gyrosigma obscurum</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Hantzschia amphioxys</i>	–	6, 11, 2	Eutrophic	Alkaliphilic	–	1,154	–	–	–	–	–

Table F-1 (continued). Diatom taxon, alternate names, reference(s), preference for Oligotrophic, Mesotrophic, or Eutrophic conditions, pH preference, and optimum soil, periphyton mat, and water total phosphorus (TP) and tolerance (tol). Taxonomic names from Jan Stevenson, Michigan State University (subject matter expert). The standard approach to obtain the weighted average optimum is to calculate the average of all the values of the particular environmental variable in which a particular species occurs, weighted by the species' relative abundance. This provides the weighted average optimum. The tolerance is the abundance-weighted standard deviation.

Taxon	Synonyms	Reference(s)	Trophic Preference	pH Preference	Trophic Indicator?	Soil TP Optima	Soil TP Tol	Mat TP Optima	Mat TP Tol	H ₂ O TP Optima	H ₂ O TP Tol
<i>Hantzschia elongata</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Hippodonta capitata</i>	—	11	Eutrophic	—	—	—	—	—	—	—	—
<i>Hippodonta hungarica</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Kobayasiella subtilissima</i>	<i>Navicula subtilissima</i>	6, 12	Mesotrophic	—	—	574	—	—	—	35	17
<i>Lemnicola hungarica</i>	<i>Achnanthes hungarica</i>	8, 12	Eutrophic	—	—	—	—	—	—	—	—
<i>Luticola mutica</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Luticola naviculoides</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Mastogloia smithii</i>	<i>Mastogloia calcarea</i>	6, 10, 12, 13	Oligotrophic	Alkaliphilic	Yes	560	307	140	96	27	15
<i>Melosira varians</i>	—	2	Eutrophic	Alkaliphilic	—	—	—	—	—	—	—
<i>Navicula arvensis</i> f. major	—	—	—	—	—	—	—	—	—	—	—
<i>Navicula atomus</i> var. <i>recondita</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Navicula cari</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Navicula cincta</i>	—	11	Eutrophic	—	—	—	—	—	—	—	—
<i>Navicula concentrica</i>	—	—	—	—	—	—	—	—	—	—	—

Table F-1 (continued). Diatom taxon, alternate names, reference(s), preference for Oligotrophic, Mesotrophic, or Eutrophic conditions, pH preference, and optimum soil, periphyton mat, and water total phosphorus (TP) and tolerance (tol). Taxonomic names from Jan Stevenson, Michigan State University (subject matter expert). The standard approach to obtain the weighted average optimum is to calculate the average of all the values of the particular environmental variable in which a particular species occurs, weighted by the species' relative abundance. This provides the weighted average optimum. The tolerance is the abundance-weighted standard deviation.

Taxon	Synonyms	Reference(s)	Trophic Preference	pH Preference	Trophic Indicator?	Soil TP Optima	Soil TP Tol	Mat TP Optima	Mat TP Tol	H ₂ O TP Optima	H ₂ O TP Tol
<i>Navicula cryptocephala</i>	–	6, 8, 12	Eutrophic	–	–	1,127	–	–	–	–	–
<i>Navicula cryptotenella</i>	–	10, 12, 3, 13	Oligotrophic - Mesotrophic	–	–	–	–	354	333	42	17
<i>Navicula difficillima</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Navicula eidrigiana</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Navicula erifuga</i>	–	11	Eutrophic	–	–	–	–	–	–	–	–
<i>Navicula exilis</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Navicula germainii</i>	–	11	Eutrophic	–	–	–	–	–	–	–	–
<i>Navicula gregaria</i>	–	11, 9	Eutrophic	–	–	–	–	–	–	54	–
<i>Navicula jaagii</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Navicula kotschyana</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Navicula lanceolata</i>	–	11, 9	Eutrophic	–	–	–	–	–	–	63	–
<i>Navicula laterostrata</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Navicula leptostriata</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Navicula libonensis</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Navicula longicephala</i>	–	–	–	–	–	–	–	–	–	–	–

Table F-1 (continued). Diatom taxon, alternate names, reference(s), preference for Oligotrophic, Mesotrophic, or Eutrophic conditions, pH preference, and optimum soil, periphyton mat, and water total phosphorus (TP) and tolerance (tol). Taxonomic names from Jan Stevenson, Michigan State University (subject matter expert). The standard approach to obtain the weighted average optimum is to calculate the average of all the values of the particular environmental variable in which a particular species occurs, weighted by the species' relative abundance. This provides the weighted average optimum. The tolerance is the abundance-weighted standard deviation.

Taxon	Synonyms	Reference(s)	Trophic Preference	pH Preference	Trophic Indicator?	Soil TP Optima	Soil TP Tol	Mat TP Optima	Mat TP Tol	H ₂ O TP Optima	H ₂ O TP Tol
<i>Navicula menisculus</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Navicula notha</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Navicula podzorskii</i>	—	12	Mesotrophic	—	—	—	—	—	—	31	16
<i>Navicula pupula</i> var. <i>aqueductae</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Navicula radiosa</i>	—	10	Oligotrophic	—	—	—	—	195	174	—	—
<i>Navicula recens</i>	—	11	Eutrophic	—	—	—	—	—	—	—	—
<i>Navicula rostellata</i>	—	11	Eutrophic	—	—	—	—	—	—	—	—
<i>Navicula</i> spp.	—	—	—	—	—	—	—	—	—	—	—
<i>Navicula stroemii</i>	—	13	Oligotrophic	—	Yes	—	—	—	—	—	—
<i>Navicula subarvensis</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Navicula submuralis</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Navicula subplacentula</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Navicula trivialis</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Navicula tugelae</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Navicula wallacei</i>	—	—	—	—	—	—	—	—	—	—	—

Table F-1 (continued). Diatom taxon, alternate names, reference(s), preference for Oligotrophic, Mesotrophic, or Eutrophic conditions, pH preference, and optimum soil, periphyton mat, and water total phosphorus (TP) and tolerance (tol). Taxonomic names from Jan Stevenson, Michigan State University (subject matter expert). The standard approach to obtain the weighted average optimum is to calculate the average of all the values of the particular environmental variable in which a particular species occurs, weighted by the species' relative abundance. This provides the weighted average optimum. The tolerance is the abundance-weighted standard deviation.

Taxon	Synonyms	Reference(s)	Trophic Preference	pH Preference	Trophic Indicator?	Soil TP Optima	Soil TP Tol	Mat TP Optima	Mat TP Tol	H ₂ O TP Optima	H ₂ O TP Tol
<i>Neidium ampliatus</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Neidium dubium</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Neidium iridis</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Nitzschia acicularioides</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Nitzschia acicularis</i>	–	11	Eutrophic	–	–	–	–	–	–	–	–
<i>Nitzschia acidoclinata</i>	–	4	Mesotrophic - Eutrophic	Circumneutral	–	–	–	–	–	–	–
<i>Nitzschia amphibia</i>	–	6	Eutrophic	–	Yes	1,303	296	1,928	1,462	70	–
<i>Nitzschia amphibia</i> f. <i>frauenfeldii</i>	–	10, 7, 12, 13	Eutrophic	–	Yes	–	–	1,560	1,964	48	16
<i>Nitzschia amphibia</i> f. <i>rostrata</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Nitzschia angustata</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Nitzschia archibaldii</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Nitzschia brevissima</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Nitzschia bulnheimiana</i>	–	–	–	–	–	–	–	–	–	–	–

Table F-1 (continued). Diatom taxon, alternate names, reference(s), preference for Oligotrophic, Mesotrophic, or Eutrophic conditions, pH preference, and optimum soil, periphyton mat, and water total phosphorus (TP) and tolerance (tol). Taxonomic names from Jan Stevenson, Michigan State University (subject matter expert). The standard approach to obtain the weighted average optimum is to calculate the average of all the values of the particular environmental variable in which a particular species occurs, weighted by the species' relative abundance. This provides the weighted average optimum. The tolerance is the abundance-weighted standard deviation.

Taxon	Synonyms	Reference(s)	Trophic Preference	pH Preference	Trophic Indicator?	Soil TP Optima	Soil TP Tol	Mat TP Optima	Mat TP Tol	H ₂ O TP Optima	H ₂ O TP Tol
<i>Nitzschia capitellata</i>	–	11, 9	Eutrophic	–	–	–	–	–	–	52	–
<i>Nitzschia clausii</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Nitzschia filiformis</i>	–	5, 11	Eutrophic	–	–	–	–	–	–	–	–
<i>Nitzschia fonticola</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Nitzschia frustulum</i>	–	6, 8, 12	Eutrophic	–	–	1,304	–	–	–	77	17
<i>Nitzschia gandersheimiensis</i>	–	12	Oligotrophic	–	–	–	–	–	–	25	13
<i>Nitzschia gessneri</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Nitzschia gracilis</i>	–	11	Eutrophic	–	–	–	–	–	–	–	–
<i>Nitzschia hantzschiana</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Nitzschia heufleriana</i>	–	9	Oligotrophic	–	–	–	–	–	–	18	–
<i>Nitzschia hamburghensis</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Nitzschia incognita</i>	–	11, 9	Oligotrophic - Eutrophic	–	–	–	–	–	–	100	–
<i>Nitzschia intermedia</i>	–	6, 11, 13	Eutrophic	–	Yes	1,296	–	–	–	–	–
<i>Nitzschia lacuum</i>	–	–	–	–	–	–	–	–	–	–	–

Table F-1 (continued). Diatom taxon, alternate names, reference(s), preference for Oligotrophic, Mesotrophic, or Eutrophic conditions, pH preference, and optimum soil, periphyton mat, and water total phosphorus (TP) and tolerance (tol). Taxonomic names from Jan Stevenson, Michigan State University (subject matter expert). The standard approach to obtain the weighted average optimum is to calculate the average of all the values of the particular environmental variable in which a particular species occurs, weighted by the species' relative abundance. This provides the weighted average optimum. The tolerance is the abundance-weighted standard deviation.

Taxon	Synonyms	Reference(s)	Trophic Preference	pH Preference	Trophic Indicator?	Soil TP Optima	Soil TP Tol	Mat TP Optima	Mat TP Tol	H ₂ O TP Optima	H ₂ O TP Tol
<i>Nitzschia liebethuthii</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Nitzschia linearis</i>	–	6	Eutrophic	–	–	1,113	–	–	–	–	–
<i>Nitzschia nana</i>	–	6, 12, 13	Eutrophic	–	Yes	1,239	–	–	–	42	10
<i>Nitzschia palea</i>	–	6, 10, 12	Mesotrophic - Eutrophic	–	–	1,359	293	313	228	57	22
<i>Nitzschia palea</i> var. <i>debilis</i>	–	10, 11, 12	Oligotrophic - Mesotrophic	–	–	–	–	254	359	–	–
<i>Nitzschia paleacea</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Nitzschia pellucida</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Nitzschia perminuta</i>	–	12	Eutrophic	–	–	–	–	–	–	58	20
<i>Nitzschia radicola</i>	–	12	Mesotrophic	–	–	–	–	–	–	48	18
<i>Nitzschia serpentiraphe</i>	–	10, 12	Oligotrophic - Mesotrophic	–	–	–	–	174	176	32	7
<i>Nitzschia sigmoidea</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Nitzschia</i> spp.	–	–	–	–	–	–	–	–	–	–	–
<i>Nitzschia subacicularis</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Nitzschia supralitorea</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Nitzschia tropica</i>	–	–	–	–	–	–	–	–	–	–	–

Table F-1 (continued). Diatom taxon, alternate names, reference(s), preference for Oligotrophic, Mesotrophic, or Eutrophic conditions, pH preference, and optimum soil, periphyton mat, and water total phosphorus (TP) and tolerance (tol). Taxonomic names from Jan Stevenson, Michigan State University (subject matter expert). The standard approach to obtain the weighted average optimum is to calculate the average of all the values of the particular environmental variable in which a particular species occurs, weighted by the species' relative abundance. This provides the weighted average optimum. The tolerance is the abundance-weighted standard deviation.

Taxon	Synonyms	Reference(s)	Trophic Preference	pH Preference	Trophic Indicator?	Soil TP Optima	Soil TP Tol	Mat TP Optima	Mat TP Tol	H ₂ O TP Optima	H ₂ O TP Tol
<i>Nitzschia vitrea</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Nupela silvahercynia</i>	—	12	Eutrophic	—	—	—	—	—	—	52	24
<i>Nupela</i> spp.	—	—	—	—	—	—	—	—	—	—	—
<i>Pinnularia acrosphaeria</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Pinnularia acuminata</i> var. <i>interrupta</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Pinnularia biceps</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Pinnularia divergens</i>	—	6	Oligotrophic	—	—	411	—	—	—	—	—
<i>Pinnularia gibba</i>	—	6, 10	Mesotrophic - Eutrophic	—	—	1,218	—	510	346	—	—
<i>Pinnularia infirma</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Pinnularia mesolepta</i> var. <i>angusta</i>	—	11	Eutrophic	—	—	—	—	—	—	—	—
<i>Pinnularia microstauron</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Pinnularia nodosa</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Pinnularia</i> spp.	—	—	—	—	—	—	—	—	—	—	—

Table F-1 (continued). Diatom taxon, alternate names, reference(s), preference for Oligotrophic, Mesotrophic, or Eutrophic conditions, pH preference, and optimum soil, periphyton mat, and water total phosphorus (TP) and tolerance (tol). Taxonomic names from Jan Stevenson, Michigan State University (subject matter expert). The standard approach to obtain the weighted average optimum is to calculate the average of all the values of the particular environmental variable in which a particular species occurs, weighted by the species' relative abundance. This provides the weighted average optimum. The tolerance is the abundance-weighted standard deviation.

Taxon	Synonyms	Reference(s)	Trophic Preference	pH Preference	Trophic Indicator?	Soil TP Optima	Soil TP Tol	Mat TP Optima	Mat TP Tol	H ₂ O TP Optima	H ₂ O TP Tol
<i>Pinnularia stomatophora</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Pinnularia subcapitata</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Pinnularia viridiformis</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Pinnularia viridis</i>	—	6	Mesotrophic	—	—	988	—	—	—	—	—
<i>Placoneis clementis</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Placoneis exigua</i>	—	11	Eutrophic	—	—	—	—	—	—	—	—
<i>Placoneis gastrum</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Planothidium rostratum</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Planothidium</i> spp.	—	—	—	—	—	—	—	—	—	—	—
<i>Platessa hustedtii</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Psammothidium marginulatum</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Psammothidium</i> spp.	—	—	—	—	—	—	—	—	—	—	—
<i>Pseudostaurosira brevistriata</i>	—	11	Eutrophic	—	—	—	—	—	—	—	—

Table F-1 (continued). Diatom taxon, alternate names, reference(s), preference for Oligotrophic, Mesotrophic, or Eutrophic conditions, pH preference, and optimum soil, periphyton mat, and water total phosphorus (TP) and tolerance (tol). Taxonomic names from Jan Stevenson, Michigan State University (subject matter expert). The standard approach to obtain the weighted average optimum is to calculate the average of all the values of the particular environmental variable in which a particular species occurs, weighted by the species' relative abundance. This provides the weighted average optimum. The tolerance is the abundance-weighted standard deviation.

Taxon	Synonyms	Reference(s)	Trophic Preference	pH Preference	Trophic Indicator?	Soil TP Optima	Soil TP Tol	Mat TP Optima	Mat TP Tol	H ₂ O TP Optima	H ₂ O TP Tol
<i>Rhoicosphenia abbreviata</i>	–	11, 9	Eutrophic	–	–	–	–	–	–	58	–
<i>Rhopalodia brebissonii</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Rhopalodia gibba</i>	–	6, 10, 12	Eutrophic	Acidophilic - Alkaliphilic	Yes	1,114	–	964	839	54	16
<i>Sellaphora bacillum</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Sellaphora laevisissima</i>	<i>Navicula laevisissima</i>	10, 11, 12	Oligotrophic - Eutrophic	–	–	–	–	324	314	–	–
<i>Sellaphora minima</i>	<i>Navicula minima</i>	6, 5, 11, 8	Eutrophic	Alkaliphilic	Yes	1,278	–	–	–	–	–
<i>Sellaphora pupula</i>	<i>Navicula pupula</i>	6, 11	Eutrophic	–	–	1,043	–	–	–	–	–
<i>Sellaphora pupula</i> var. <i>rectangularis</i>	<i>Navicula pupula</i> var. <i>rectangularis</i>	8, 2	Mesotrophic - Eutrophic	–	–	–	–	–	–	–	–
<i>Sellaphora seminulum</i>	<i>Navicula seminulum</i>	6, 11, 12, 13	Eutrophic	–	Yes	1,210	–	–	–	57	19
<i>Sellaphora</i> spp.	–	–	–	–	–	–	–	–	–	–	–
<i>Sellaphora stroemii</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Stauroneis anceps</i>	–	–	–	–	–	–	–	–	–	–	–

Table F-1 (continued). Diatom taxon, alternate names, reference(s), preference for Oligotrophic, Mesotrophic, or Eutrophic conditions, pH preference, and optimum soil, periphyton mat, and water total phosphorus (TP) and tolerance (tol). Taxonomic names from Jan Stevenson, Michigan State University (subject matter expert). The standard approach to obtain the weighted average optimum is to calculate the average of all the values of the particular environmental variable in which a particular species occurs, weighted by the species' relative abundance. This provides the weighted average optimum. The tolerance is the abundance-weighted standard deviation.

Taxon	Synonyms	Reference(s)	Trophic Preference	pH Preference	Trophic Indicator?	Soil TP Optima	Soil TP Tol	Mat TP Optima	Mat TP Tol	H ₂ O TP Optima	H ₂ O TP Tol
<i>Stauroneis anceps</i> f. <i>gracilis</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Stauroneis kriegei</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Stauroneis obtusa</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Stauroneis phoenicenteron</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Stauroneis producta</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Stauroneis prominula</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Stauroneis pseudosubobtusoides</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Stenopterobia curvula</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Stephanodiscus medius</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Synedra acus</i>	—	12, 2, 13	Eutrophic	Alkaliphilic	Yes	—	—	—	—	56	24
<i>Synedra acus</i> var. <i>angustissima</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Synedra biceps</i>	—	—	—	—	—	—	—	—	—	—	—
<i>Synedra delicatissima</i> var. <i>angustissima</i>	—	11, 2	Oligotrophic - Mesotrophic	—	—	—	—	—	—	—	—

Table F-1 (continued). Diatom taxon, alternate names, reference(s), preference for Oligotrophic, Mesotrophic, or Eutrophic conditions, pH preference, and optimum soil, periphyton mat, and water total phosphorus (TP) and tolerance (tol). Taxonomic names from Jan Stevenson, Michigan State University (subject matter expert). The standard approach to obtain the weighted average optimum is to calculate the average of all the values of the particular environmental variable in which a particular species occurs, weighted by the species' relative abundance. This provides the weighted average optimum. The tolerance is the abundance-weighted standard deviation.

Taxon	Synonyms	Reference(s)	Trophic Preference	pH Preference	Trophic Indicator?	Soil TP Optima	Soil TP Tol	Mat TP Optima	Mat TP Tol	H ₂ O TP Optima	H ₂ O TP Tol
<i>Synedra dilatata</i>	–	–	–	–	–	–	–	–	–	–	–
<i>Synedra rumpens</i>	–	2	Oligotrophic - Eutrophic	–	–	–	–	–	–	–	–
<i>Synedra ulna</i>	<i>Fragilaria ulna</i> <i>Ulnaria ulna</i>	6, 8, 12, 2	Eutrophic	Alkaliphilic	Yes	948	–	–	–	57	22

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Appendix G. History of Pilot Sampling

Whelan, K. R. T., and R. Urgelles. 2017. History of pilot sampling. South Florida/Caribbean Network. Palmetto Bay, Florida.

Introduction

The South Florida/Caribbean Inventory and Monitoring Network (SFCN) conducted a series of pilot studies to assist periphyton monitoring protocol development that will allow for assessment of effects from anthropogenic disturbances (altered hydroperiod and water quality) and comparison to other regional monitoring currently underway. Following is a description of pilot monitoring conducted from 2008–2014. Data are summarized in Table G-1.

Table G-1. Pilot sampling summary of purpose, types of analyses conducted, and number of samples taken in different basins. BCR= Birdon Road Culverts; BH=Black Hole; BI=Bear Island; EC=East Crossing Strand; EH=East Hinson Marsh; FP=Fire Prairie; KB=Kissimmee Billy Strand (Little Marsh); KBE=Kissimmee Billy East; MN=Monument; MT=Monument Trail; OK=Okaloacoochee Slough.

Sample Dates	# Samples	Purpose	TP	Diatom	Soft Algae	BI	EC	EH	FP	MN	OK	KB	KBE	MT	BH	BCR
Dec 8-10, 2008	41	(1) determine if both diatom and soft algae metrics were needed or if diatoms alone would suffice, (2) determine sufficient differentiation between impacted and unimpacted sites to use periphyton as a long-term indicator of ecologically impacted sites, and (3) determine if community structure differed significantly between diatom/soft algae assemblages found in cypress domes and those found in immediately adjacent prairie marshes.	–	Yes	Yes	4	1	3	1	3	4	–	–	2	–	–
Nov. 17-10, 2009	65	(1) provide better understanding of nutrient gradient, as depicted through diatom communities, across the hydrological basins in NW BICY	–	Yes	–	–	9	9	10	8	7	10	–	–	–	–
Nov. 22- Dec. 7, 2010	36	(1) detect any temporal variability in the structure of diatom communities at sites that had been repeatedly sampled	–	Yes	–	–	6	5	6	5	5	6	–	–	–	–
Jan. 12- 26, 2012	40	(1) to detect any significant temporal shifts in diatom community structure at the collection sites, and (2) to expand sampling to the east by adding a seventh basin: Kissimmee Billy East (KBE). (3) To investigate proposed new sites for BICY water-quality collection.	–	Yes	–	1	5	5	5	5	6	5	5	–	1	–
Nov 28-Dec 5, 2012	46	(1) Continue the development of the temporal periphyton signal in previously sampled sites as well as the proposed new water-quality sites	–	Yes	–	1	8	5	7	5	6	7	5	–	1	–

Table G-1 (continued). Pilot sampling summary of purpose, types of analyses conducted, and number of samples taken in different basins. BCR= Birdon Road Culverts; BH=Black Hole; BI=Bear Island; EC=East Crossing Strand; EH=East Hinson Marsh; FP=Fire Prairie; KB=Kissimmee Billy Strand (Little Marsh); KBE=Kissimmee Billy East; MN=Monument; MT=Monument Trail; OK=Okaloacoochee Slough.

Sample Dates	# Samples	Purpose	TP	Diato m	Soft Algae	BI	EC	EH	FP	MN	OK	KB	KBE	MT	BH	BCR
Nov 5-21, 2013	48	(1) add another basin, Bear Island (between impacted basins OK Slough and East Hinson Marsh and unimpacted basin Kissimmee Billy Strand), to provide a better understanding of a nutrient gradient, and (2) to collect a second set of periphyton samples from the sites and analyze them for mat TP content.	Yes	Yes	–	5	8	5	6	5	7	7	5	–	–	–
Dec 1-5, 2014	56	(1) continue the development of the temporal periphyton signal in previously sampled sites, and (2) collect a second set of samples from the sites to analyze them for mat TP content.	Yes	Yes	–	5	9	7	8	7	8	9	–	–	–	3

December 2008

We conducted our pilot sampling from December 8–10, 2008. Forty-one periphyton samples were collected from eight basins in the northwest portion of Big Cypress National Preserve. At the time of this sampling, not all the necessary information was available to provide proper delineation for all basins. Also, due to access limitations, widespread sampling throughout each basin was not possible. However, our main goals were to (1) determine if both diatom and soft algae metrics were needed or if diatoms alone would suffice; (2) determine sufficient differentiation between impacted and unimpacted sites using periphyton as a long-term indicator of ecologically impacted sites, and; (3) determine if community structure differed significantly between diatom/soft algae assemblages found in cypress domes and those found in immediately adjacent prairie marshes.

Site selection was based on accessibility from a road or off-road vehicle (ORV) trail and on flow characteristics. Travel to sites was done in part by a motorized four-wheel drive vehicle and later by foot. Sampling at each site took place at paired dome and marsh “community-types.” Grab samples were preserved in a 3% buffered formalin solution upon collection in the field and sent to Dr. R. Jan Stevenson at Michigan State University for sample processing and community analyses. The Stevenson lab processed the samples for diatom and soft algae content and created an interpretive report of the data that indicated the ecological condition of the collection site based on the taxonomic grouping and other relevant published work.

November 2009

We conducted our second sampling of periphyton in Big Cypress National Preserve from November 17–19, 2009. The goal of the second-year sampling was to provide a better understanding of a nutrient gradient, as depicted through diatom communities, across the hydrological basins in the northwest portion of the preserve. As a result, the number of periphyton collection sites was increased for the 2009 sampling in order to cover as much of each basin as possible. This increase in sample size would potentially indicate any variability in the diatom community, from a spatial perspective, within each basin.

We sampled in broadleaf marshes whenever possible since this type of habitat retains water for most of the year. Site selection was based on the following criteria: broadleaf marsh habitat, flow characteristics, spatial distribution, proximity to an ORV trail, and safe for a helicopter to land. All sites were accessed by either helicopter or all-terrain vehicles (ATVs). Sixty-five periphyton mat samples were collected from sites in six hydrological basins. Samples were preserved and sent to Dr. R. Jan Stevenson at Michigan State University for diatom identification and community analyses.

November 2010

We conducted our third sampling of periphyton in Big Cypress National Preserve from November 22–December 7, 2010. The goal of the third-year sampling was to detect any temporal variability in the structure of diatom communities at sites repeatedly sampled. Periphyton mat samples again collected from sites in six hydrological basins; three nutrient-impacted and three unimpacted, topographically defined by watershed boundaries and flow direction. The sample size was decreased from the previous year’s collection (36 samples in 2010 as opposed to 65 samples in 2009) due to financial constraints. Sites within the basins were accessed by helicopter and by ATVs.

January 2012

The fourth sampling of periphyton in Big Cypress National Preserve took place from January 12–26, 2012. Periphyton sample collection took place later than usual in the 2011–2012 water year due to high water levels in the month of November, limiting accessibility to most of the sites. The main goals of the fourth-year sampling were:

- To detect any significant temporal shifts in diatom community structure at the collection sites.
- To expand sampling to the east by adding a seventh basin: Kissimmee Billy East (i.e. KBE).
- To investigate proposed new sites for Big Cypress National Preserve water-quality collection.

Kissimmee Billy East basin was delineated and sampled due to a continuous high total phosphorus signature in the water column, indicated by the basin's hydro station. Forty samples were collected: 33 from sites previously sampled in 2009 and 2010, five from the newly added basin, and two from locations proposed by Big Cypress National Preserve staff for future water-quality monitoring stations. Sites within the basins were accessed by helicopter and by all-terrain vehicles (ATVs).

November 2012

From November 28 to December 5, 2012, the South Florida/Caribbean Network conducted its fifth sampling for periphyton in Big Cypress National Preserve. The main goal of the fifth-year sampling was to continue the development of the temporal periphyton signal in previously sampled sites as well as the proposed new water-quality sites. Periphyton samples were collected from 46 sites consecutively sampled for the past three years, including three locations proposed by Big Cypress National Preserve staff for future water-quality monitoring stations. Collection sites were located within seven designated basins and were accessed by helicopter and ATVs. Samples were preserved in 3% formalin and sent to Dr. R. Jan Stevenson at Michigan State University for diatom counts and identification.

November 2013

The South Florida/Caribbean Network conducted its sixth sampling from November 5–21, 2013. The main goals for this year were to: (1) add another basin, Bear Island (between impacted basins Okaloacoochee Slough and East Hinson Marsh and unimpacted basin Kissimmee Billy Strand), to provide a better understanding of a nutrient gradient, and (2) to collect a second set of periphyton samples from the sites and analyze them for mat TP content.

Sites in Bear Island basin demonstrate diatom assemblages that fall in between those of unimpacted sites (e.g., Kissimmee Billy Strand and East Crossing Strand basins) and those of impacted sites (e.g., Okaloacoochee Slough and East Hinson Marsh basins). Thus, a nutrient gradient can be observed and allow the network a better understanding of TP thresholds for diatom assemblages.

Periphyton mat total phosphorus results from the November 2013 sampling corroborated the results for site diatom assemblage. Total phosphorus concentrations in the mat explain nearly 80% of diatom species composition in ordination analysis that matches biological data with environmental data.

December 2014

In December 2014, the South Florida/Caribbean Network sampled sites in each of the seven basins, totaling 56 sites. In addition to the regularly sampled sites, sites sampled only once before were sampled again this season in order to investigate diatom communities and TP content. This is the second TP data collection so we can begin to understand the temporal pattern to this data set. The TP data will create a better understanding of a nutrient gradient across northwest Big Cypress National Preserve and help establish TP thresholds for diatom communities. The Kissimmee Billy East basin was not sampled this year as the past three years of data indicated an unimpacted diatom community. Additional sites were added to the more recently sampled Bear Island basin to characterize a broader area therein. Two samples were collected at each site; one for diatom assessment, and one for TP. We added three collection sites in the proposed Birdon Road Culvert rehydration site in order to have a baseline to compare against future data.

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