Aquatic Site Sampling Design – NEON Domain 03

PREPARED BY	ORGANIZATION	DATE
Stephanie Parker	AQU	1/23/2019
Nora Catolico	AQU	1/23/2018
Keli Goodman	AQU	5/26/2016
Brandon Jensen	AQU	11/3/2016
Charlotte Roehm	AQU	
Jenna Stewart	AQU	

APPROVALS	ORGANIZATION	APPROVAL DATE
Kate Thibault	SCI	04/04/2019
Mike Stewart	PSE	04/06/2019
David Barlow	ENG	05/22/2019

RELEASED BY	ORGANIZATION	RELEASE DATE
Anne Balsley	CM	05/23/2019

See configuration management system for approval history.

The National Ecological Observatory Network is a project solely funded by the National Science Foundation and managed under cooperative agreement by Battelle. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
Historic changes			CM updated with new template and changes based on riparian habitat assessment timing; Updated site access location maps with correct well names; add spatial tables; Updated riparian assessment coordinates with FOPs data; Updated bathymetry timing; Updated site maps; Updated bio contingencies and water chem sampling locations in seepage lakes
A	05/23/2019	ECO-06095	Initial release

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

1 DESCRIPTION

1.1 Purpose

The goal of the National Ecological Observatory Network (NEON) is to enable understanding and forecasting of the impacts of climate change, land use change, and invasive species on continental-scale ecology.

A disparity exists in the scale of organisms and their effects on the global environment (Hargrove & Pickering, 1992). While environmental impacts often occur at the largest scales, small scale biological and physical processes need to be understood in order to document responses of organisms, communities, populations and other small scale phenomena (Keller et al., 2008). Data will be gathered from the level of gene to ecosystem at a local to continental scale using standardized field procedures and sample processing. In order to address this disparity, NEON will approach the Grand Challenge questions through an analysis of processes, interactions and responses occurring across spatial and temporal scales.

The local data collected at NEON sites within the 20 Domains will be integrated with the targeted regional data from NEON airborne instrumentation. This will provide a direct linkage in spatial and temporal scaling from NEON's distributed sensor network and in-situ field measurements, coupled with individual plant or canopy measurements to plot or stand level observations, and ultimately to the continental scale.

1.2 Scope

This document outlines the Domain 03 site-specific sampling strategy proposed for NEON Aquatic field sampling activities and other directly associated activities that will be used to address key data products related to the overarching Grand Challenge questions. It provides the sampling rationale for given parameters.

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

Applicable documents contain information that shall be applied in the current document. Examples are higher level requirements documents, standards, rules and regulations.

AD [01]	NEON.DOC.000001	NEON Observatory Design
AD [02]	NEON.DOC.002652	NEON Level 1, Level 2, Level 3 Data Products Catalog
AD [03]	NEON.DOC.005011	NEON Coordinate Systems Specification

2.2 Reference Documents

Reference documents contain information complementing, explaining, detailing, or otherwise supporting the information included in the current document.

RD [01]	NEON.DOC.000008	NEON Acronym List
RD [02]	NEON.DOC.000243	NEON Glossary of Terms
RD [03]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD [04]	NEON.DOC.001085	AOS Protocol and Procedure: Stream Discharge
RD [05]	NEON.DOC.001197	AOS Protocol and Procedure: Bathymetry and Morphology of Lakes and Non-Wadeable Streams
RD [06]	NEON.DOC.002905	AOS Protocol and Procedure: Water Chemistry Sampling in Surface Waters and Groundwater
RD [07]	NEON.DOC.001886	AOS Protocol and Procedure: Stable Isotope Sampling in Surface and Ground Waters
RD [08]	NEON.DOC.001199	AOS Protocol and Procedure: Surface Water Dissolved Gas Sampling
RD [09]	NEON.DOC.001191	AOS Protocol and Procedure: Sediment Chemistry Sampling in Lakes and Non-Wadeable Streams
RD [10]	NEON.DOC.003044	AOS Protocol and Procedure: Aquatic Microbe Sampling
RD [11]	NEON.DOC.003045	AOS Protocol and Procedure: Periphyton, Seston, and Phytoplankton Sampling
RD [12]	NEON.DOC.003039	AOS Protocol and Procedure: Aquatic Plant, Bryophyte, Lichen, and Macroalgae Sampling
RD [13]	NEON.DOC.003046	AOS Protocol and Procedure: Aquatic Macroinvertebrate Sampling
RD [14]	NEON.DOC.001194	AOS Protocol and Procedure: Zooplankton Sampling in Lakes
RD [15]	NEON.DOC.003826	AOS Protocol and Procedure: Riparian Habitat Assessment
RD [16]	NEON.DOC.001296	AOS Protocol and Procedure: Fish Sampling in Lakes
RD [17]	NEON.DOC.004613	NEON Preventative Maintenance Procedure: AIS Buoy

2.3 Acronyms

C0-C3	Buoy sensor set
IN	Inlet sensor set
OT	Outlet sensor set
GDD	Growing degree days
MGC	Multivariate geographic clustering
MODIS	Moderate Resolution Imaging Spectroradiometer
NOAA	National Oceanic and Atmospheric Administration
NCDC (NCEI)	National Centers for Environmental Information
S1	Aquatic reach sensor set 1
S2	Aquatic reach sensor set 2

3 TEMPORAL SAMPLING STRATEGY

3.1 Rationale

NEON designed a set of domains based on a statistically rigorous analysis using national data sets for eco-climatic variables, based upon algorithms for multivariate geographic clustering (MGC) (Hargrove & Hoffman, 1999, 2004). The MGC approach identified nine primary climate state variables that could define

the domains, allowing for regionalization of primary features within each domain. In order to replicate the strategy used for the large scale spatial design of NEON, Aquatics has adapted this approach and modified the list of the nine variables by identifying variables that were equally pertinent to the large scale temporal design, and by adding critical variables that affect physical, biological and chemical parameters in aquatic environments.

Aquatic ecosystems exhibit physical, chemical and biological variability over a wide range of spatial and temporal scales (Steele, 1978). This has resulted in a movement towards research approaches that utilize concurrent field based, buoy, aircraft, and satellite sampling strategies in order to measure physical, chemical and biological distributions over large areas synoptically and over long time periods. The integration of such sampling strategies across scales is an integral part of NEON's approach to the addressing the Grand Challenge questions (Keller et al., 2008).

NEON must be able to extrapolate relationships between drivers (climate change, land use change, and biological invasions) and ecological consequences to areas that are not sampled by NEON facilities but where partial, extensively sampled, or gridded information is available. In order to obtain this NEON's temporal sampling strategy must be equally designed to detect and quantify trends over time, as well as characterizing the spatial pattern of those trends. The sampling approach at the field scale, hence, must address the temporal.

3.2 Approach

Sampling strategies must cover a range of temporal scales and must address issues of duration and frequency of sampling activities. The design of the temporal strategy for NEON Aquatics addresses both the duration and frequency of the field activities as well as the small scale but long-term continuous monitoring data collection. In addition, prioritization of the physical, biological and chemical parameters needs to be identified. The general layout of a NEON lake site are presented in Section 6.

NEON Aquatics has proposed the following approach in order to determine the sampling duration and frequency that will yield the best estimate of composition and/or concentration of the physical, biological and chemical parameters (Table 1, Table 2). No fish sampling will occur at Domain 03 lake sites at this time due to the presence of alligators.

Physical/Chemical: Air temperature has been identified as the main variable defining the timing and frequency of sampling for physical and chemical parameters. Air temperature controls the dynamics of ice-on and ice-off events as well as stratification and turnover events.

Biological: Degree days, water temperature, and riparian greenness are the primary variables identified for defining the timing and frequency of sampling of most biological parameters.

Sampling modules may also have specific rule sets that dictate the order and timing of collection, as well as time constraints on laboratory work to maintain viable samples. The rule sets below (Table 3, Table 4) have been identified for specific sampling modules.

Table 1. Duration, frequency and prioritization of field activities and long term monitoring for NEON lake sites as a function of targeted constraints and driving variables. For associated lab hours, see Appendix A. (*May be scheduled more frequently if a stochastic event significantly alters the lake basin.) Fish sampling is not currently planned at D03 lakes due to the presence of alligators.

Sampling Module	Sampling Duration (hrs)	Sampling Frequency (x per year)	Constraints on Sampling	Driving Metrics for Sampling	Priority
Sensor Maintenance					
Surface water	1-2	26	Water Temperature Discharge	None	High
Meteorological	1-2	26	Weather	None	High
Groundwater (light)	1-2	26	Weather	None	High
Groundwater (full)	2-4	4	Weather	None	High
Well redevelopment	4	1	Weather	None	High
Physical					
Bathymetry	8-40	1 per 5 yrs*	Wind Ice-off	Riparian greenness	Low to Medium
Biological					
Surface Microbes	2-4	6	Ice-off Wind	Precipitation Water Temperature	High
Aquatic plants and Macroalgae	3-8	3	Ice-off Wind	Precipitation Light (PAR)	High
Macroinvertebrates	3	3	Ice-off Wind	Precipitation Water Temperature	High
Zooplankton	3	3	Ice-off Wind	Precipitation Water Temperature	High
Periphyton and phytoplankton	3	3	Ice-off Wind	Precipitation Light (PAR)	High
Riparian habitat assessment	2-4	1	Wind	Riparian greenness	Low
Chemical					
Surface water chemistry	1-3	12	Ice-off Wind	Precipitation Water Temperature	High
Dissolved gas	1	12	Ice-off Wind	Precipitation Water Temperature	Medium
Isotopes	2	12	Ice-off Precipitation	Precipitation Water Temperature	High
Sediment chemistry	4-8	2	Ice-off Wind	Flow Regime Water Temperature	Low to medium
Groundwater chemistry	8-20	2	Sufficient Water in Well	Groundwater Elevation, Seasonal (spring, fall)	Medium to High

Table 2. Duration, frequency and prioritization of field activities and long term monitoring for NEON river sites as a function of targeted constraints and driving variables. For associated lab hours, see Appendix B. (*May be scheduled more frequently if a stochastic event significantly alters channel morphology.)

Sampling Module	Sampling Duration (hrs)	Sampling Frequency (x per year)	Constraints on Sampling	Driving Metrics for Sampling	Priority
Sensor Maintenance					

Surface water	1-2	26	Water Temperature Discharge	None	High
Meteorological	1-2	26	Weather	None	High
Groundwater (light)	1-2	26	Weather	None	High
Groundwater (full)	2-4	4	Weather	None	High
Well redevelopment	4	1	Weather	None	High
Physical					
River discharge	1-3	12	Flow	Flow Regime Precipitation	High
Bathymetry	8-32	1 per 5 yrs*	Flow Wind	Riparian greenness Flow Regime	Low to Medium
Biological					
Surface microbes	2-4	6	Discharge	Flow Regime Water Temperature	High
Aquatic plants and Macroalgae	3-8	3	Discharge	Flow Regime Light (PAR)	High
Macroinvertebrates	3	3	Discharge	Flow Regime Water Temperature	High
Periphyton and phytoplankton	3	3	Discharge	Flow Regime Light (PAR)	High
Riparian habitat assessment	4-8	1	Discharge	Riparian greenness	Low
Chemical					
Surface water chemistry	1-3	26	Discharge	Flow Regime Water Temperature	High
Dissolved gas	1	26	Discharge	Flow Regime Water Temperature	Medium
Isotopes	2	26	Precipitation	Flow Regime Water Temperature	High
Sediment chemistry	4-8	2	Discharge	Flow Regime Water Temperature	Low to medium
Groundwater chemistry	8-20	2	Sufficient Water in Well	Flow Regime	Medium to High

Table 3. Rule sets for sampling modules in lakes. Deviations may be allowed with science approval. Fish sampling is not currently planned at D03 lakes due to the presence of alligators.

Protocol	Rule set
Water chemistry, dissolved gas, and isotopes	Should be completed first to reduce the risk of contamination. However, if completing multiple protocols that could take more than a few hours, collect chemistry samples last to reduce the time between collection and processing/shipping.
	Collect recurrent samples on Tuesdays, when possible.
	Alkalinity/ANC lab processing must begin within 24 hours of collection, or the sample must be flagged.
Surface water microbes	Sample in conjunction with recurrent (usually Tuesday) water chemistry.
	Filters must be flash-frozen in the field, and kept frozen until storage in -80 °C freezer. If processing in the domain lab, freeze at -80 °C within 4 hours of collection.
	Cell counts must be preserved in the field. Maximum time to preservation if bad weather = 4 hours.
Aquatic plants	Lab processing must begin within 48 hours of collection, or the sample must be flagged. AFDM samples may be dried and placed in desiccators until enough room is available in the muffle furnace.
	Biomass collection (clip harvest) only occurs during Bout 2.
Macroinvertebrates	Must be preserved within 1 hour of collection.
	Preservative change must occur within 12-72 hours of collection.
Zooplankton	Must be preserved with 30 minutes of collection.
Periphyton/Phytoplankton	Lab processing must begin within 24 hours of collection. AFDM samples may be dried and placed in desiccators until enough room is available in the muffle furnace. Minimum lab processing time spans 2 days.
	Sample must be kept cool (~4 °C) and dark until processing at the domain lab.
	Chlorophyll filters must be shipped to the external facility within 7 days of collection.
Sediment chemistry	Start field collection after non-fish biological sampling to minimize disturbance.
Bathymetry	Bathymetry occurs every 5 years unless extreme events warrant more frequent surveys.
	Bathymetric mapping occurs at peak greenness, during Bio Bout 2 or within ± 2 weeks of aquatic plant sampling.
Riparian habitat assessment	Riparian habitat assessment must occur during peak greenness.
Groundwater Chemistry	Completed within ± 1 day of water chemistry (contingency situations may necessitate 2 days).
Well redevelopment	Must not occur in the 2 weeks prior to groundwater chemistry sampling.

Table 4. Rule sets for sampling modules in rivers. Deviations may be allowed with science approval.

Protocol	Rule set
Water chemistry, dissolved gas, and isotopes	Should be completed first to reduce the risk of contamination. However, if completing multiple protocols that could take more than a few hours, collect chemistry samples last to reduce the time between collection and processing/shipping.
	Collect recurrent samples on Tuesdays, when possible.
	Alkalinity/ANC lab processing must begin within 24 hours of collection, or the sample must be flagged.
Surface water microbes	Sample in conjunction with recurrent (usually Tuesday) water chemistry.
	Filters must be flash-frozen in the field, and kept frozen until storage in -80 °C freezer. If processing in the domain lab, freeze at -80 °C within 4 hours of collection.
	Cell counts must be preserved in the field. Maximum time to preservation if bad weather = 4 hours.
Aquatic plants	Lab processing must begin within 48 hours of collection, or the sample will be flagged. AFDM samples may be dried and placed in desiccators until enough room is available in the muffle furnace.
	If a flood occurs, wait a minimum of 5 days after water level drops below 3x median discharge to allow for macroalgal recolonization.
	Biomass collection (clip harvest) only occurs during Bout 2.
Macroinvertebrates	If a flood occurs, wait a minimum of 5 days after water level drops below 3x median discharge to allow for recolonization.
	Must be preserved within 1 hour of collection.
	Preservative change must occur within 12-72 hours of collection.
Periphyton, Phytoplankton	If a flood occurs, wait a minimum of 14 days after the water level drops below 3x median discharge to allow the periphyton community to recolonize.
	Lab processing must begin within 24 hours of collection. AFDM samples may be dried and placed in desiccators until enough room is available in the muffle furnace. Minimum lab processing time spans 2 days.
	Sample must be kept cool (~4 °C) and dark until processing at the domain lab.
	Chlorophyll filters must be shipped to the external facility within 7 days of collection.
Sediment chemistry	If a flood occurs, wait 5 days before sampling to allow sediments to settle. If water clarity improves and the presence of depositional zones occur in less than 5 days, sediment sampling may resume.
	Start field collection after non-fish biological sampling to minimize disturbance.
Bathymetry	Bathymetry occurs every 5 years unless extreme events warrant more frequent surveys.
	Bathymetric mapping occurs during the peak greenness window, either during Bio Bout 2 or within ± 2 weeks of aquatic plant sampling.
Riparian habitat assessment	Riparian habitat assessment must occur during peak greenness.
Groundwater Chemistry	Completed within ± 1 day of water chemistry (contingency situations may necessitate 2 days).
Well redevelopment	Must not occur in the 2 weeks prior to groundwater chemistry sampling.
Discharge	A wide range of flow conditions should be targeted for measurement throughout the water year given safety considerations.

4 SAMPLING DATES

The surface water sampling strategy for the D03 lake sites (Barco and Suggs) is based on annual air temperature data collected from NOAA National Climatic Data Center (NCDC) and from the near-real time NEON data collected at the meteorological stations. Because these sites are ice-free throughout the year, surface water samples can be collected on a monthly basis, while organismal sampling is based on accumulation of growing degree days throughout the year.

The surface water sampling strategy for Flint River is based on hydrological data collected at a nearby Flint River USGS gauging station. However, hydrologic conditions may dictate a need to be updated the strategy following annual data collections.

The following Tables and Figures indicate proposed sampling dates for all sample protocols to be undertaken at Domain 03 over the course of a year.

4.1 Sensor Maintenance

Sensor preventative maintenance for in-lake/river sensors and the meteorological station is scheduled every other week. Groundwater well maintenance includes light sensor maintenance every other week (confirm that the cables have not slipped, check for ice accumulation on the solar panel, remotely monitor the data stream), full maintenance quarterly (visually inspect the sensor, check the desiccant, check water clarity with bailers, check for roots in wells known to have that issue), and well redevelopment once per year. Additional details may be found in the preventative maintenance documents for each sensor and the lake and river buoys (RD[17]).

4.2 Surface and Groundwater Chemistry Sampling Dates

4.2.1 Water Chemistry Sampling Dates – BARC, SUGG

Water chemistry includes sampling for water chemistry, aquatic stable isotopes, and dissolved gas in surface waters. These protocols should be completed on the same day as each other at each site.

Alkalinity and ANC titrations: Following a minimum of a year of alkalinity and ANC titrations at lake inflow, center (buoy), and outflow, it was determined that no significant difference existed between the three lake locations. Thus, we will only complete alkalinity and ANC titrations from the buoy location.

Standard recurrent sampling should take place 12 times per year on every first Tuesday of the month starting on the first Tuesday of the year, in coordination with TIS chemistry sampling and other national programs to enable standardization. Due to proximity of sites, surface water chemistry samples may be taken on the same day at both Lake Barco and Lake Suggs.

4.2.2 Groundwater Chemistry Sampling Dates – BARC, SUGG

Groundwater chemistry includes sampling for water chemistry and aquatic stable isotopes (^{2}H and ^{18}O-H_2O only). These protocols should be completed on the same day as each other at each site.

Groundwater samples will be collected twice per year from a subset of 4 wells per site (Table 5). The wells will be specified prior to sampling and will remain the same between bouts. Two wells are sampled on the inlet side, and two on the outlet side of the lake. This will allow for chemical comparisons at opposite ends of the regional flow paths in addition to lake surface water samples.

The range of groundwater sampling dates, shown in Table 6 has been selected to target one sampling event in the spring and one in the autumn conditions. The main constraint on groundwater sampling is to couple this event within two days (preferably 1 day) of a lake chemistry-sampling bout. This constraint aims to clarify origin of chemical fluxes between upstream sources versus local groundwater sources to the surface water by tightly coupling in time the two sampling bouts. The date range is provided to allow flexibility for Field Ops in selecting a time within the sampling event window where both sampling bouts can be performed in a maximum of a three-day period. Dates will be refined after the first few years of site-specific water table data are available for analysis.

Table 5. Groundwater Observation Wells at D03 Suggs and Barco. **Wells for groundwater chemistry sampling are denoted in bold text.**

BARC Well ID	Latitude	Longitude
D03-BARC-OW-01	**29.6785743**	**82.0085794**
D03-BARC-OW-02	29.6779559	82.0076001
D03-BARC-OW-03	29.6768872	82.0066929
D03-BARC-OW-04	29.6748150	82.0066619
D03-BARC-OW-05	**29.6740463**	**82.0071731**
D03-BARC-OW-06	**29.6740680**	**82.0082592**
D03-BARC-OW-07	29.6748107	82.0104955
D03-BARC-OW-08	**29.6780496**	**82.0091775**
SUGG Well ID	**Latitude**	**Longitude**
D03-SUGG-OW-01	29.6938797	82.0183457
D03-SUGG-OW-02	29.6931475	82.0189623
D03-SUGG-OW-03	29.6932174	82.0172479
D03-SUGG-OW-04	**29.6913313**	**82.0157809**
D03-SUGG-OW-05	**29.6889941**	**82.0141058**
D03-SUGG-OW-06	**29.6836649**	**82.0152635**
D03-SUGG-OW-07	29.6847159	82.0150605
D03-SUGG-OW-08	**29.6844424**	**82.0176792**

Table 6. Proposed groundwater chemistry sampling dates for D03 Suggs and Barco.

BARC, SUGG Well Bout	Start Date	End Date
1	February 20	March 20
2	September 20	October 20

4.2.3 Water Chemistry Sampling Dates – FLNT

Standard recurrent sampling should take place one Tuesday per month, 12 times per year, in coordination with atmospheric chemistry sampling. The remaining 14 samples should be taken based on the cumulative discharge of the stream representing the increasing and decreasing periods of annual peak flow (Figure 1; Table 7). The 14 samples should be collected within 2-3 days of all proposed sampling dates, when possible. If circumstances dictate that you have to miss one of your flow-weighted sampling events and cannot reschedule within the 2-3 day window, you may re-schedule another sampling event up to 14 days from the proposed date. If one of the 14 flow-weighted samples falls on the same days as a monthly Tuesday sample, adjust the flow-weighted sample by ± 7 days.

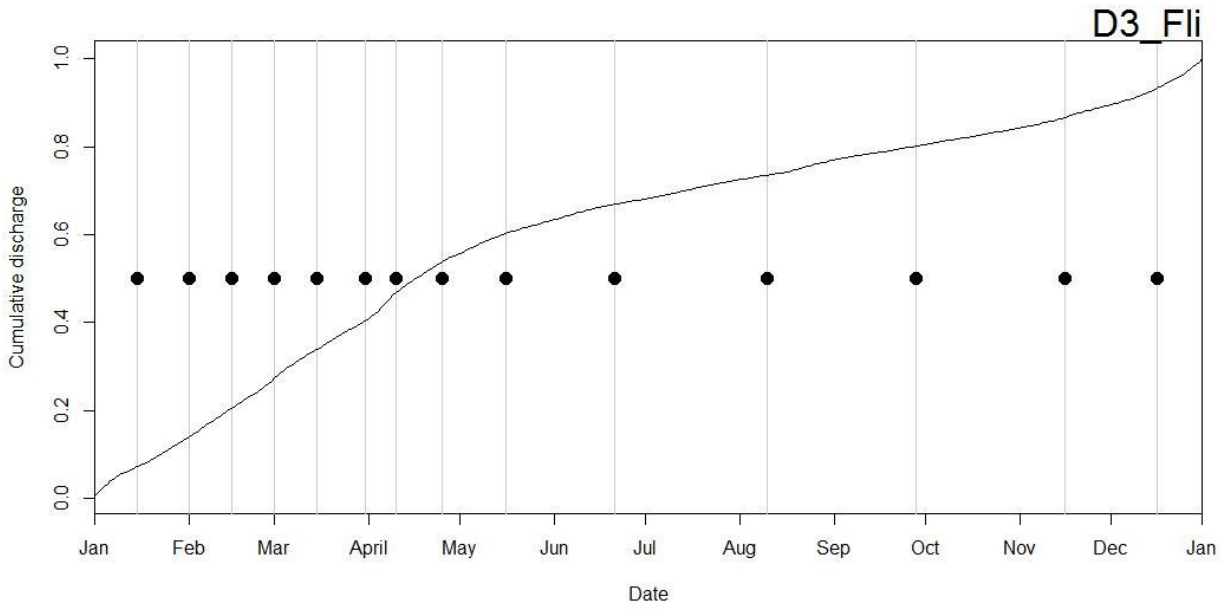


Figure 1. Timing of sample collection for 14 water chemistry samples reflecting the discharge related strategy.

Table 7. Proposed stream water chemistry sampling dates for D03 Flint River for the 14 samples collected to reflect the discharge related strategy.

FLNT Proposed sampling dates
January 15th

February 1st
February 15th
March 1st
March 15th
March 31st
April 10th
April 25th
May 16th
June 21st
August 10th
September 28th
November 16th
December 16th

4.2.4 Groundwater Chemistry Sampling Dates – FLNT

Groundwater chemistry includes sampling for water chemistry and aquatic stable isotopes (^{2}H and ^{18}O-H_2O only). These protocols should be completed on the same day as each other at each site.

Groundwater samples will be collected twice per year from a subset of 4 wells per site (Table 9). The wells will be specified prior to sampling and will remain the same between bouts. The four sampling wells are selected in attempt to cover the following categories: upstream, downstream, and near-stream. This strategy allows for more direct comparison to surface water chemistry data.

Sampling will occur between 20-30% and 70-80% of the historically available cumulative discharge curve. Dates are summarized in Table 9 and shown in relation to the cumulative discharge in Figure 2 below. Groundwater sampling should be timed to occur on the same day (preferred) or within 1-2 days (preferably 1 day) of the surface water collection. Dates will be refined after the first few years of site-specific water table data are available for analysis.

Table 8. Proposed Groundwater Observation Wells to Sample at D03 Flint River.

FLNT Well ID	Latitude	Longitude
D03-FLNT-OW-01	**31.18627**	**84.437423**
D03-FLNT-OW-02	31.185828	84.438875
D03-FLNT-OW-03	**31.185574**	**84.438204**
D03-FLNT-OW-04	31.184689	84.439214
D03-FLNT-OW-05	31.184453	84.439396
D03-FLNT-OW-06	**31.183474**	**84.440532**
D03-FLNT-OW-07	**31.182761**	**84.441471**

Table 9. Proposed groundwater chemistry sampling dates for D03 Flint River.

FLNT Well Bout	Start Date	End Date

1	February 14	March 6
2	July 14	September 27

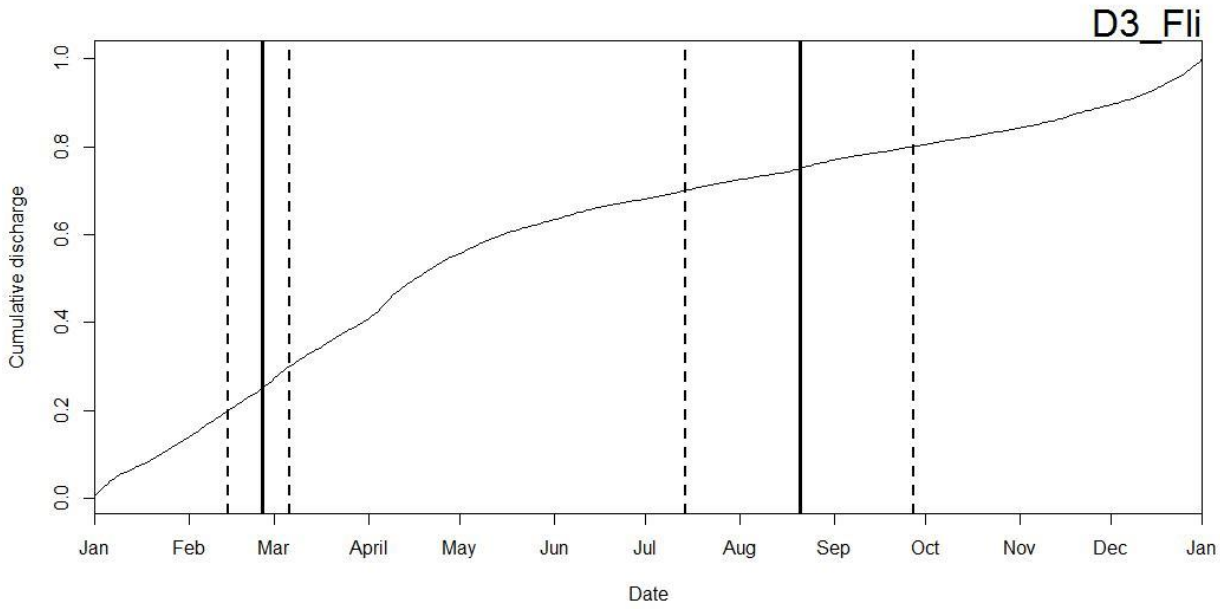


Figure 2. Timing of sample collection for 14 water chemistry samples reflecting the discharge related strategy.

4.3 Biology Bout, Sediment Chemistry Sampling, and Riparian Assessment Dates

4.3.1 Lake Barco and Lake Suggs

The biology bout windows for lakes are based on a combination of parameters at each site. Using mean air temperature (NOAA NCDC datasets) to calculate growing degree days (centering around 10%, 50%, and 90% gdd) and the MODIS dataset to estimate riparian greenness (green-up and brown-down; Figure 3, Figure 4), 1 month sampling windows were pre-determined for all sites. Sampling windows may be adjusted for ice off dates as all biological sampling (except for surface water microbes) is based on actual conditions at the site. The riparian assessment will be conducted during the site-specific peak greenness window defined by the MODIS dataset which may or may not coincide with the biological and sediment sampling bout dates (Table 10).

- Surface water microbes should be sampled in conjunction with the standard recurrent water chemistry sampling every other month, 6 times per year on every first Tuesday of the month starting on the first Tuesday of the year.
    - Surface water DNA microbe samples collected during July or August should be marked for metagenomics analysis.

- Sampling for all other biological modules (aquatic plants/macroalgae, macroinvertebrates, zooplankton, phytoplankton/periphyton) as well as sediment chemistry, follow pre-determined sampling windows are presented in Table 10. Sediment chemistry is sampled twice per year during Bouts 1 and 3.
    - The biology/sediment chemistry bout windows may be adjusted to start 3 days earlier or and/or end 3 days later than the dates listed in Table 10 to allow for more flexibility in scheduling. Any sampling outside of the bout window plus the 3-day buffer will require an entry in NEON's problem-tracking system.
    - Sampling for each module at a site must occur within one day, with the exception of bathymetry which may take up to 5 days at a site.
- The riparian habitat assessment will occur within the dates provided in Table 10.

Table 10. Proposed Biological sampling windows in D03 Lakes Barco and Suggs. Sediment Chemistry will take place during Bouts 1 and 3. The riparian habitat assessment peak greenness window may not coincide with the bout windows.

SUGG, BARC Bio Bout	Start Dates	End Dates
1	February 9	March 9
2	June 27	July 25
3	October 29	November 26
Riparian	June 25	September 28

4.3.1.1 Suggested Biology and Sediment Chemistry Bout – BARC, SUGG

1. Aquatic plants, macroinvertebrates, periphyton/phytoplankton, zooplankton (in any order)
    a. Secchi/Depth profile data collection must also occur on days when phytoplankton and zooplankton are sampled.
2. Sediment chemistry (Bouts 1 and 3 only)

4.3.1.2 Other Biology Sampling – BARC, SUGG

- Surface water microbes – 1st water chemistry bout of every-other month (likely Tuesday)
- Bathymetry – schedule within ± 2 weeks of Bout 2 aquatic plant sampling
    - Occurs every 5 years unless morphology changes significantly due to an extreme event
- The riparian habitat assessment can be scheduled anytime within the peak greenness window

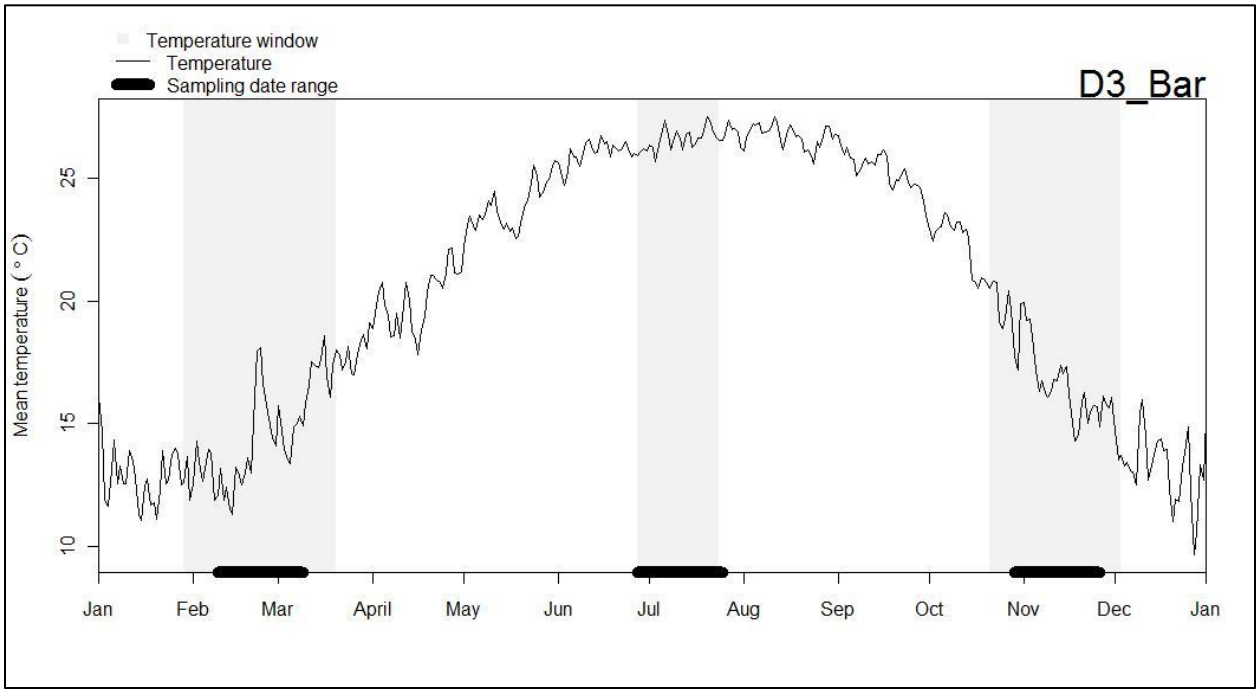


Figure 3. Proposed bouts for biological sampling at Lake Barco and Lake Suggs. Sediment chemistry occurs during Bouts 1 and 3.

4.3.2 Flint River

The biology bout windows for rivers are based on a combination of parameters at each site. Using USGS streamflow data from nearby "proxy" sites, mean daily air temperature (NOAA NCDC datasets) to calculate growing degree days (centering around 10%, 50%, and 90% gdd) and the MODIS dataset to estimate riparian greenness (green-up and brown-down; Figure 4), 1 month sampling windows were pre-determined for all sites. Sampling windows may be adjusted for high-flow dates as all biological sampling (except for surface water microbes) is based on actual conditions at the site. The riparian assessment will be conducted during the site-specific peak greenness window defined by the MODIS dataset which may or may not coincide with the biological and sediment sampling bout dates (Table 11).

Sampling at FLNT should only be conducted when conditions are safe for boating. Domain 03 Field Science estimates that it is unsafe to boat at the FLNT site when discharge is greater than 12,000 cfs as determined by the upstream USGS gaging station (K. Ludwig, *pers. comm.*)

- Surface water microbes should be sampled in conjunction with the standard recurrent water chemistry sampling every other month, 6 times per year on every first Tuesday of the month starting on the first Tuesday of the year.
    - Surface water DNA microbe samples collected during July or August should be marked for metagenomics analysis.

- Sampling windows for biology and sediment chemistry bouts are presented in Table 11. Sediment chemistry is sampled twice per year during Bouts 1 and 3. Fish are not sampled by NEON at river sites.
    - The biology/sediment chemistry bout windows may be adjusted to start 3 days earlier or and/or end 3 days later than the dates listed in Table 11 to allow for more flexibility in scheduling. Any sampling outside of the bout window plus the 3-day buffer will require an entry in NEON's problem-tracking system.
    - Sampling for each module at a site must occur within one day, with the exception of bathymetry.
- The riparian habitat assessment will occur within the dates provided in Table 11.

Table 11. Proposed Biological sampling windows in D03 Flint River. Bathymetry will be conducted during Bout 2. Sediment chemistry will be sampled during Bouts 1 and 3. The riparian habitat assessment peak greenness window may not coincide with the bout windows.

FLNT Bio Bout	Start Dates	End Dates
1	February 21	March 21
2	June 27	July 25
3	October 7	November 4
Riparian	July 17	September 2

4.3.2.1 Suggested Biology and Sediment Chemistry Bout – FLNT

1. Aquatic plants, macroinvertebrates, periphyton/phytoplankton (in any order)
    a. Secchi/Depth profile data collection must also occur on days when phytoplankton and zooplankton are sampled.
2. Sediment chemistry (Bouts 1 and 3 only)

4.3.2.2 Other Biology Sampling – FLNT

- Surface water microbes – 1st water chemistry bout of every-other month (likely Tuesday)
- Bathymetry – schedule within ± 2 weeks of Bout 2 aquatic plant sampling
    - Occurs every 5 years unless morphology changes significantly due to an extreme event
- The riparian habitat assessment can be scheduled anytime within the peak greenness window

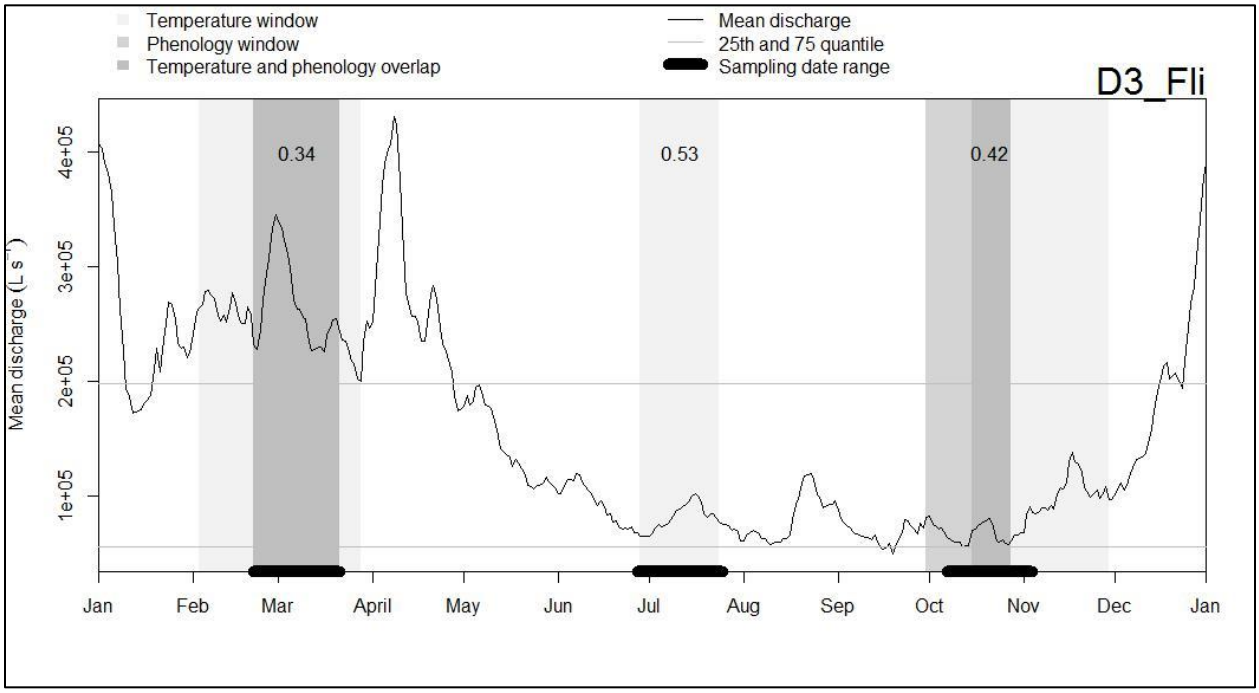


Figure 4. Proposed bouts for biological sampling at Flint River. Sediment chemistry will be sampled during Bouts 1 and 3.

5 PROTOCOL DISTURBANCE AND PRIORITIZATION

5.1 Disturbance Criteria

Each aquatic protocol has its own unique sensitivity to disturbance and perturbations (Table 12). These sensitivities should dictate the order in which protocols are completed.

Table 12. Disturbance Criteria for lake and river sampling. Impact level: high (4), medium/high (3), medium/low (2), low (1), none (0). Bathymetry/morphology spans the entire permitted area. Sensors are located at the deepest point in the lake, and near the lake inlet and outlet. Fish sampling does not occur in rivers or in D03 lakes due to alligators, and wading is unsafe in SUGG and FLNT.

Sample	Requirements	Impact Level	Disturbance
Sensor maintenance	None	1	Boat activity near sensor locations
Bathymetry	None	1	Boat activity throughout lake
Aquatic plants	None	3	Benthic collection at randomized points throughout the lake
Invertebrates	None	3	Benthic collection near water chemistry sampling sites and wading and substrate disturbance near shore
Zooplankton	6 hours- no disturbance that causes turbid conditions	2	Boat activity near sensor locations

Sample	Requirements	Impact Level	Disturbance
Periphyton and phytoplankton	6 hours- no disturbance that causes turbid conditions	2	Wading and substrate disturbance near shore, boat activity near sensor locations
Sediment chemistry	None	4	Boat activity and benthic disturbance near sensor locations
Surface water chemistry, dissolved gas, isotopes, and surface microbes	None	1	Boat activity near sensor locations
Groundwater chemistry	None	0	Groundwater Removal
Riparian habitat assessment	None	2	Boat activity and substrate disturbance nearshore

6 SPATIAL SAMPLING STRATEGY

6.1 General Site Sampling Locations

Lake sampling protocols reference several locations within the lake, including the buoy (or center) location, the inlet sensor, and the outlet sensor (i.e., water chemistry, isotopes, dissolved gas, surface water microbes, phytoplankton, zooplankton protocols). Lake sites are also divided into 10 sections divided by the riparian bank locations (Figure 5), which are used when sampling macroinvertebrates, periphyton, and the riparian habitat assessment. Locations provided in the "proposed" columns in Table 15 and Table 16 are estimates. Specific coordinates at each site will be used for the life of the site.

River sampling protocols reference several locations within the river, including the sensor set location and riparian transect locations (Figure 6). See Table 17 for module-specific sampling locations and rule sets, and Figure 5 and Figure 6 for generic site layouts.

Table 13. Module-specific sampling locations, lakes.

Sampling module	Location
Surface water chemistry	Buoy and groundwater wells
Dissolved gas	Buoy
Isotopes	Buoy and groundwater wells
Surface water microbes	Buoy
Phytoplankton	Buoy, lake inlet sensor (adjusted in-lake location for bio sampling), lake outlet sensor (adjusted in-lake location for bio sampling)
Riparian habitat assessment	Sections determined by HQ and provided to domain staff
Periphyton	In riparian sections, exact location determined by field ecologists
Aquatic plants, bryophytes, and macroalgae	10 randomized points
Macroinvertebrates (ponar)	Buoy, lake inlet sensor (adjusted in-lake location for bio sampling), lake outlet sensor (adjusted in-lake location for bio sampling)
Macroinvertebrates (sweep)	In riparian sections, exact location determined by field ecologists
Zooplankton	Buoy, lake inlet sensor (adjusted in-lake location for bio sampling), lake outlet sensor (adjusted in-lake location for bio sampling)

Sampling module	**Location**
Sediment chemistry	Buoy, lake inlet sensor (adjusted in-lake location for bio sampling)
Groundwater wells	Locations determined by HQ
Bathymetry	Whole lake

Table 14. Module-specific sampling locations, rivers.

Sampling module	**Location**
Surface water chemistry	Buoy and groundwater wells
Dissolved gas	Buoy
Isotopes	Buoy and groundwater wells
Surface water microbes	Buoy
Phytoplankton	Buoy and locations in reach determined by field ecologists
Riparian habitat assessment	Transects determined by HQ and provided to domain staff
Periphyton	In riparian sections, exact location determined by field ecologists
Aquatic plants, bryophytes, lichens, and macroalgae	10 random points
Macroinvertebrates (ponar)	Buoy and locations in reach determined by field ecologists
Macroinvertebrates (sweep)	In riparian sections, exact location determined by field ecologists
Sediment chemistry	Two 250 m stations are established with in the 500 m sediment sampling reach. Sediment is collected from depositional zones within each station which may change bout to bout.
Groundwater wells	Locations determined by HQ
Bathymetry	Whole reach

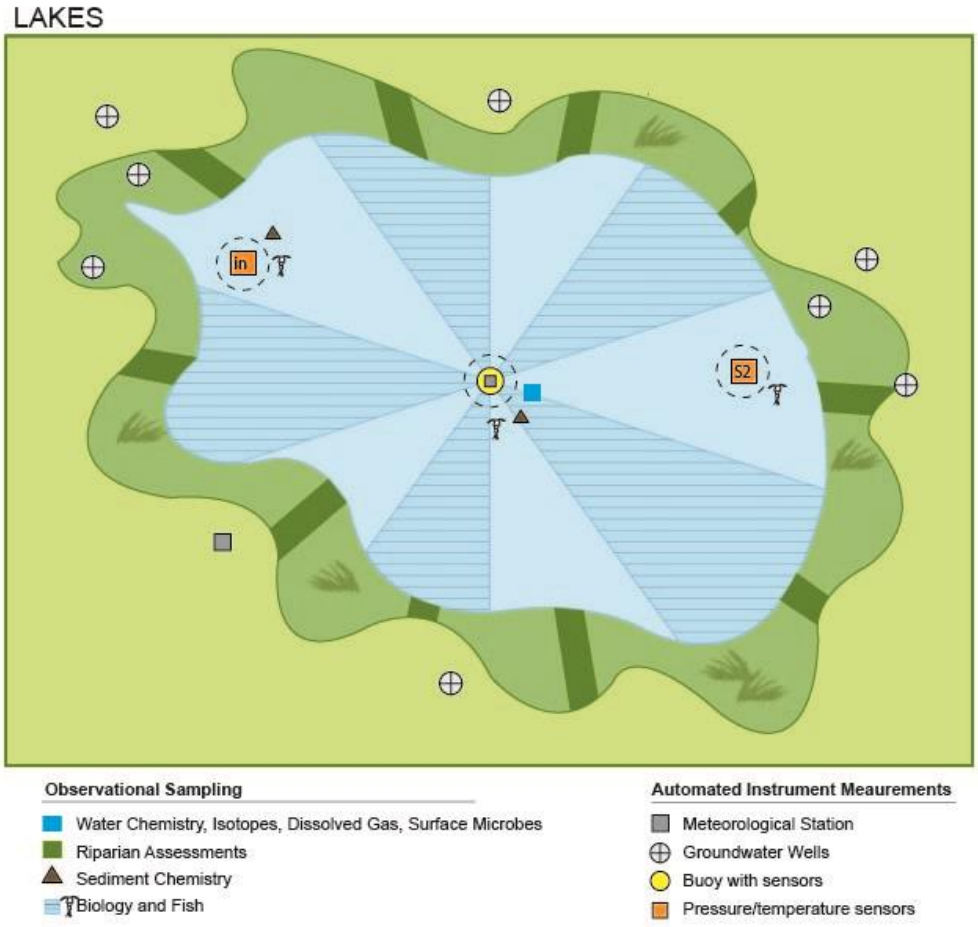


Figure 5 General diagram for an AQU site showing sampling locations in a seepage lake system.

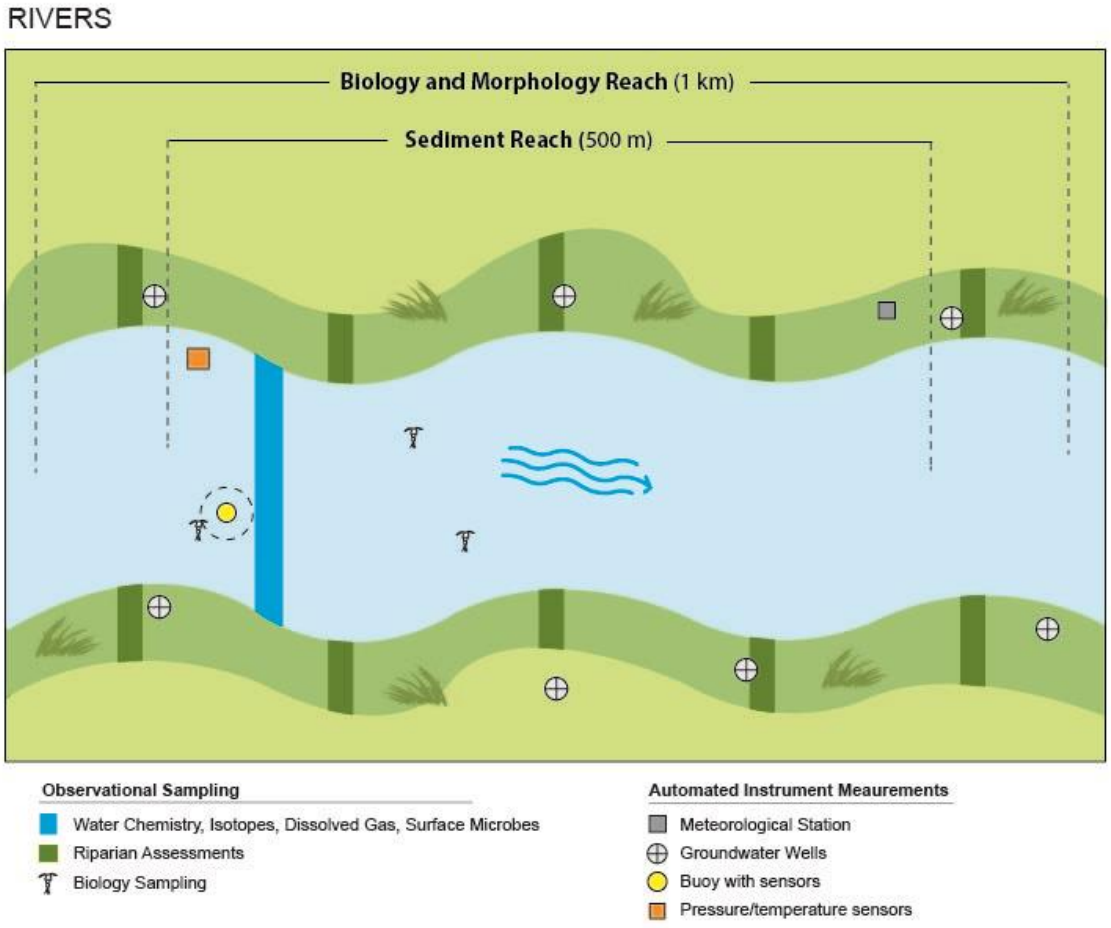


Figure 6. General diagram for an AQU site showing sampling locations in a river system. Sediment stations 1 and 2 are divided by the biological reach center.

Table 15. BARC Sampling Locations. Proposed coordinates are determined prior to sampling at HQ. Coordinates are groundtruthed by Field Science in the field and reported to Science. If available in the table, Field Science coordinates should be used for sampling.

Location ID	*Description*	*Proposed Latitude*	*Proposed Longitude*	*Field Sci Latitude*	*Field Sci longitude*
01 - Riparian	Riparian coordinates*	29.677514	-82.008487	29.677778	-82.007670
02 - Riparian	Riparian coordinates*	29.677250	-82.007526	29.677034	-82.006975
03 - Riparian	Riparian coordinates*	29.676490	-82.007004	29.676118	-82.006975
04 - Riparian	Riparian coordinates*	29.675565	-82.006956	29.675164	-82.006743
05 - Riparian	Riparian coordinates*	29.674888	-82.007573	29.674476	-82.007454
06 - Riparian	Riparian coordinates*	29.674838	-82.0086	29.674507	-82.008596
07 - Riparian	Riparian coordinates*	29.675119	-82.009617	29.674953	-82.009818
08 - Riparian	Riparian coordinates*	29.675639	-82.010254	29.675584	-82.010729
09 - Riparian	Riparian coordinates*	29.676399	-82.00977	29.676597	-82.009967
10 - Riparian	Riparian coordinates*	29.676977	-82.009247	29.677686	-82.008910
Inlet	Inlet location from SCR	29.677519	-82.008153	29.677480	-82.007723
Outlet	Outlet location from SCR	29.674985	-82.008728	29.674754	-82.008407
C0 (buoy)	C0 sensor location from SCR	29.676139	-82.008445		

*Riparian coordinates should be approximately evenly spaced throughout the sampling area

Table 16. SUGG Sampling Locations. Proposed coordinates are determined prior to sampling at HQ. Coordinates are groundtruthed by Field Science in the field and reported to Science. If available in the table, Field Science coordinates should be used for sampling. Riparian markers are not used at SUGG due to swampy banks.

Location ID	*Description*	*Proposed Latitude*	*Proposed Longitude*	*Field Sci Latitude*	*Field Sci longitude*
01 - Riparian	Riparian coordinates*	29.69038	-82.019605		
02 - Riparian	Riparian coordinates*	29.690679	-82.017404		
03 - Riparian	Riparian coordinates*	29.689972	-82.015337		
04 - Riparian	Riparian coordinates*	29.688129	-82.014426		
05 - Riparian	Riparian coordinates*	29.686477	-82.014568		
06 - Riparian	Riparian coordinates*	29.685435	-82.016359		
07 - Riparian	Riparian coordinates*	29.684758	-82.018516		
08 - Riparian	Riparian coordinates*	29.685231	-82.020373		
09 - Riparian	Riparian coordinates*	29.687067	-82.020473		
10 - Riparian	Riparian coordinates*	29.688825	-82.020784		
Inlet	Inlet location from SCR	29.685093	-82.018412	29.685105	-82.018411
Outlet	Outlet location from SCR	29.687588	-82.020613	29.687597	-82.020616
C0 (buoy)	C0 sensor location from SCR	29.688176	-82.017373		

* Riparian coordinates should be approximately evenly spaced throughout the sampling area

Table 17. FLNT Sampling Locations. Proposed coordinates are determined prior to sampling at HQ. Coordinates are groundtruthed by Field Science in the field and reported to Science. If available in the table, Field Science coordinates should be used for sampling. Riparian markers are not installed at FLNT due to steep banks (points 1-5) and private property (points 6-10).

Location ID	*Description*	*Proposed Latitude*	*Proposed Longitude*	*Field Sci Latitude*	*Field Sci longitude*
01 - Riparian	Riparian coordinates*	31.182529	-84.441583		
02 - Riparian	Riparian coordinates*	31.183951	-84.439516		
03 - Riparian	Riparian coordinates*	31.185639	-84.437721		
04 - Riparian	Riparian coordinates*	31.187253	-84.435923		
05 - Riparian	Riparian coordinates*	31.189125	-84.434547		
06 - Riparian	Riparian coordinates*	31.188701	-84.433661		
07 - Riparian	Riparian coordinates*	31.186885	-84.435329		
08 - Riparian	Riparian coordinates*	31.185068	-84.437045		
09 - Riparian	Riparian coordinates*	31.183373	-84.438931		
10 - Riparian	Riparian coordinates*	31.181911	-84.441051		
permitted top	Top of permitted reach**	31.189125	-84.434547		
permitted bottom	Bottom of permitted reach**	31.182529	-84.441583		
TOP	Top of sampling reach***	31.189125	-84.434547		
BOT	Bottom of sampling reach***	31.182529	-84.441583		
C0 (buoy)	S1 sensor location from SCR	31.184565	-84.438431		

*Riparian coordinates should be approximately evenly spaced throughout the sampling reach

**Do not sample outside of this boundary

***Should be 1000 m unless permitting restricted

6.2 Site Access and Instrument Locations

NEON sites will be visited by field ecologists on a regular basis. To protect the environment near sites, several access points have been established at each site to minimize local disturbance over the life of the site at locations (e.g., sensors and boat launches) that are accessed frequently (Figure 7, Figure 8, and Figure 9. Field ecologists must use established paths and access points when possible to avoid causing disturbance to the site.

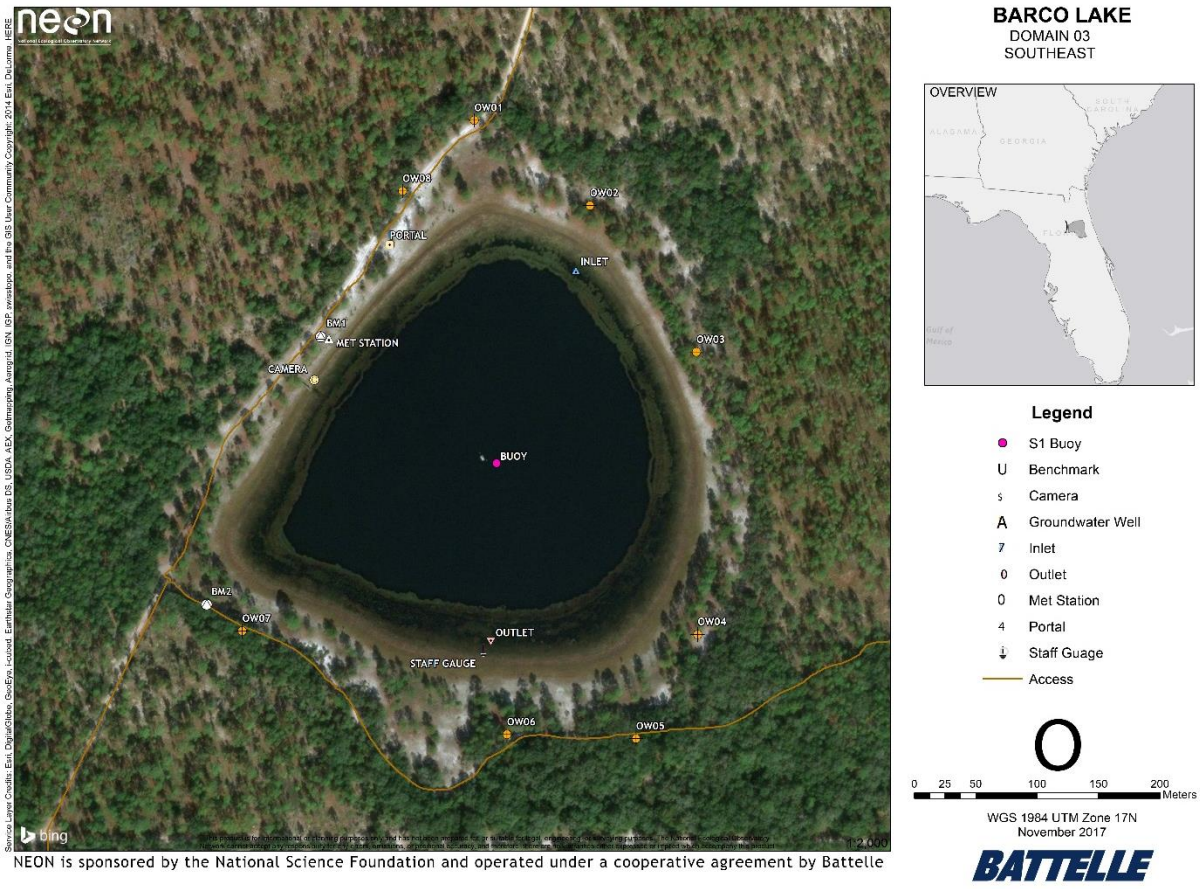


Figure 7. Site access and instrument locations at D03 Lake Barco

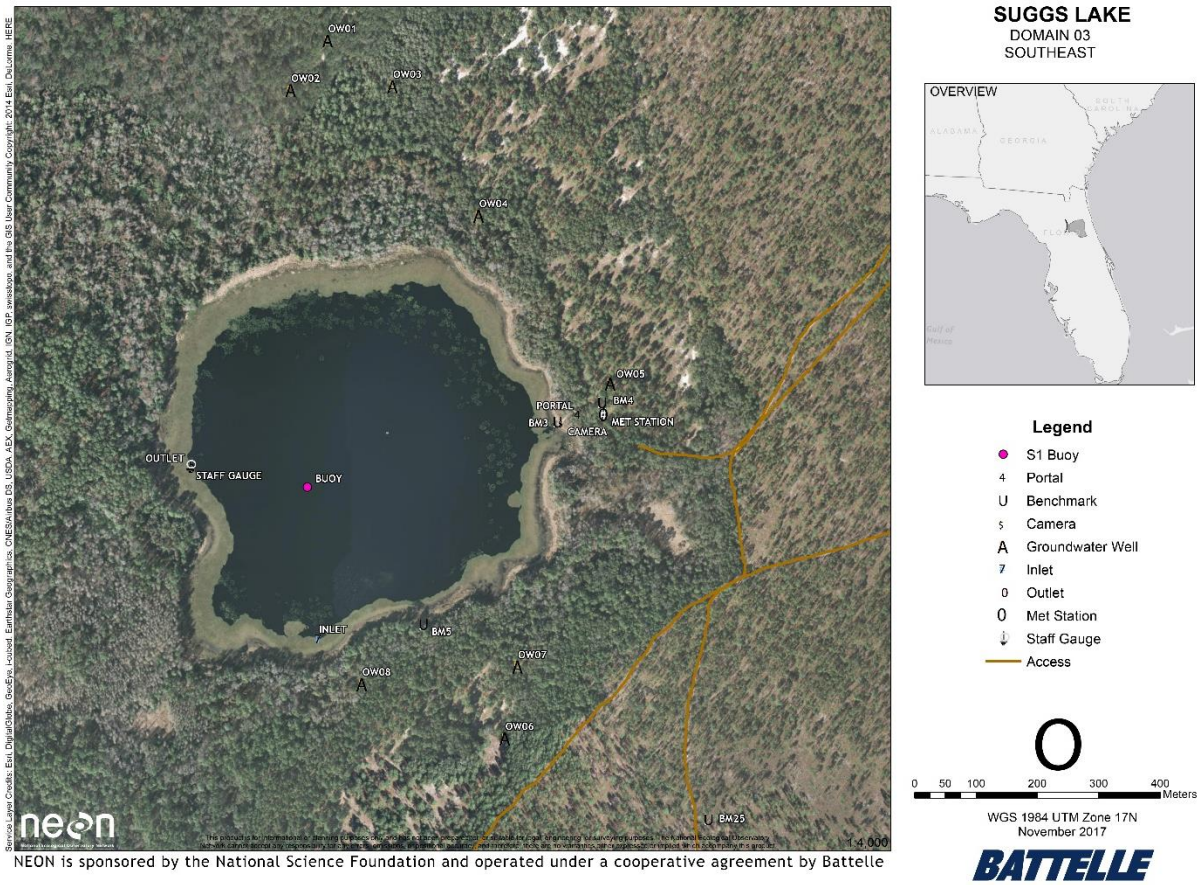


Figure 8. Site access and instrument locations at D03 Lake Suggs

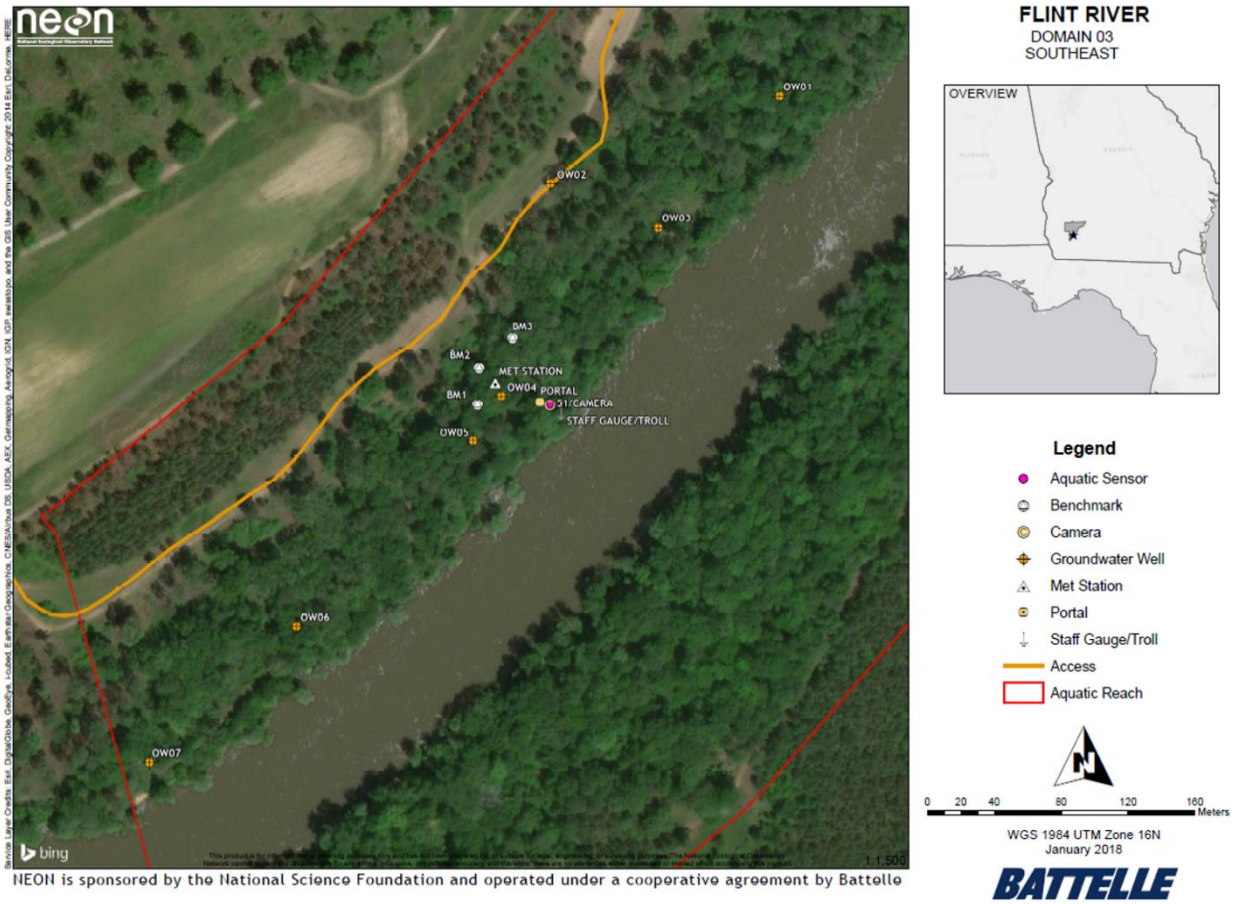


Figure 9. Site access and instrument locations at Flint River.

6.3 Riparian Sampling Locations

Riparian coordinates are determined prior to sampling at HQ. Coordinates at most sites are groundtruthed by Field Science (exceptions: SUGG and FLNT) in and reported back to Science to update Table 15, Table 16, Table 17. If available in the table, Field Science coordinates should be used for riparian sampling. Lake riparian sections are numbered from 1-10 clockwise around the lake (Figure 10, Figure 11). River riparian sections are numbered from 1-10 starting at the river right downstream end of the permitted reach, and numbered clockwise around the reach (Figure 12).

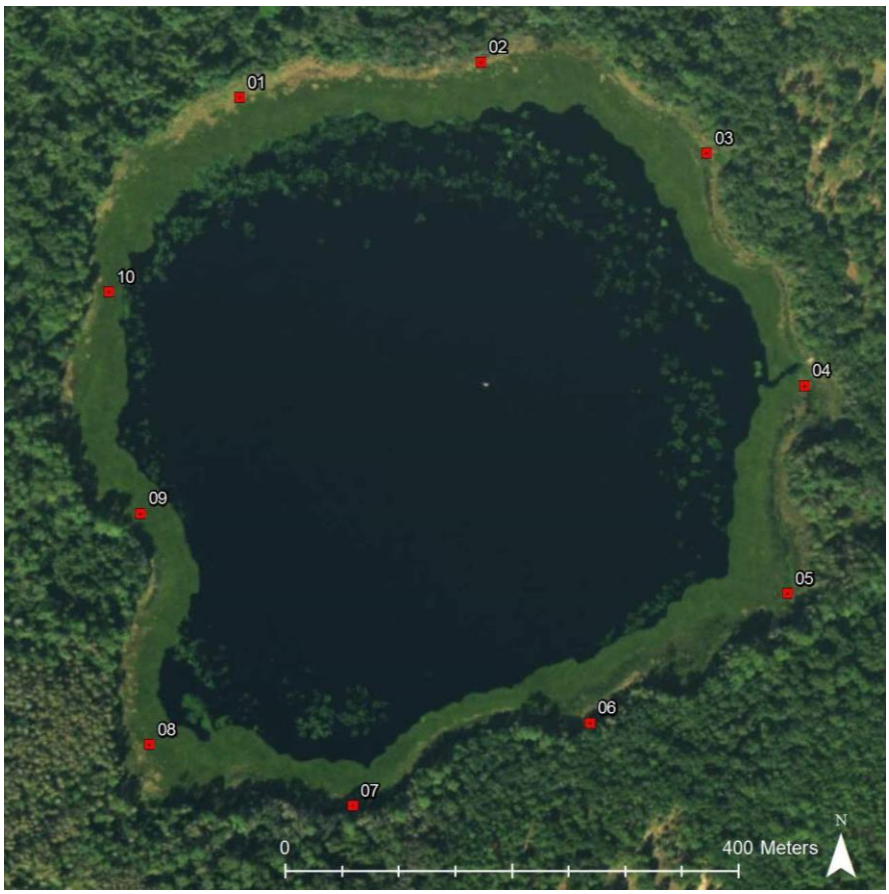


Figure 10. SUGG ideal riparian sampling design

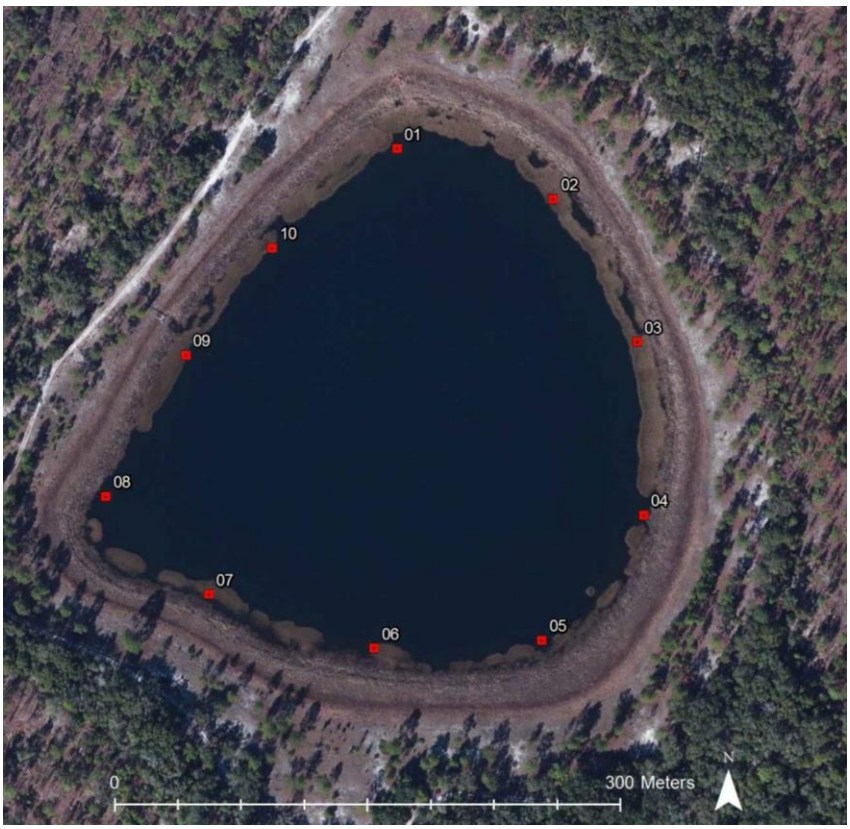


Figure 11. BARC ideal riparian sampling design

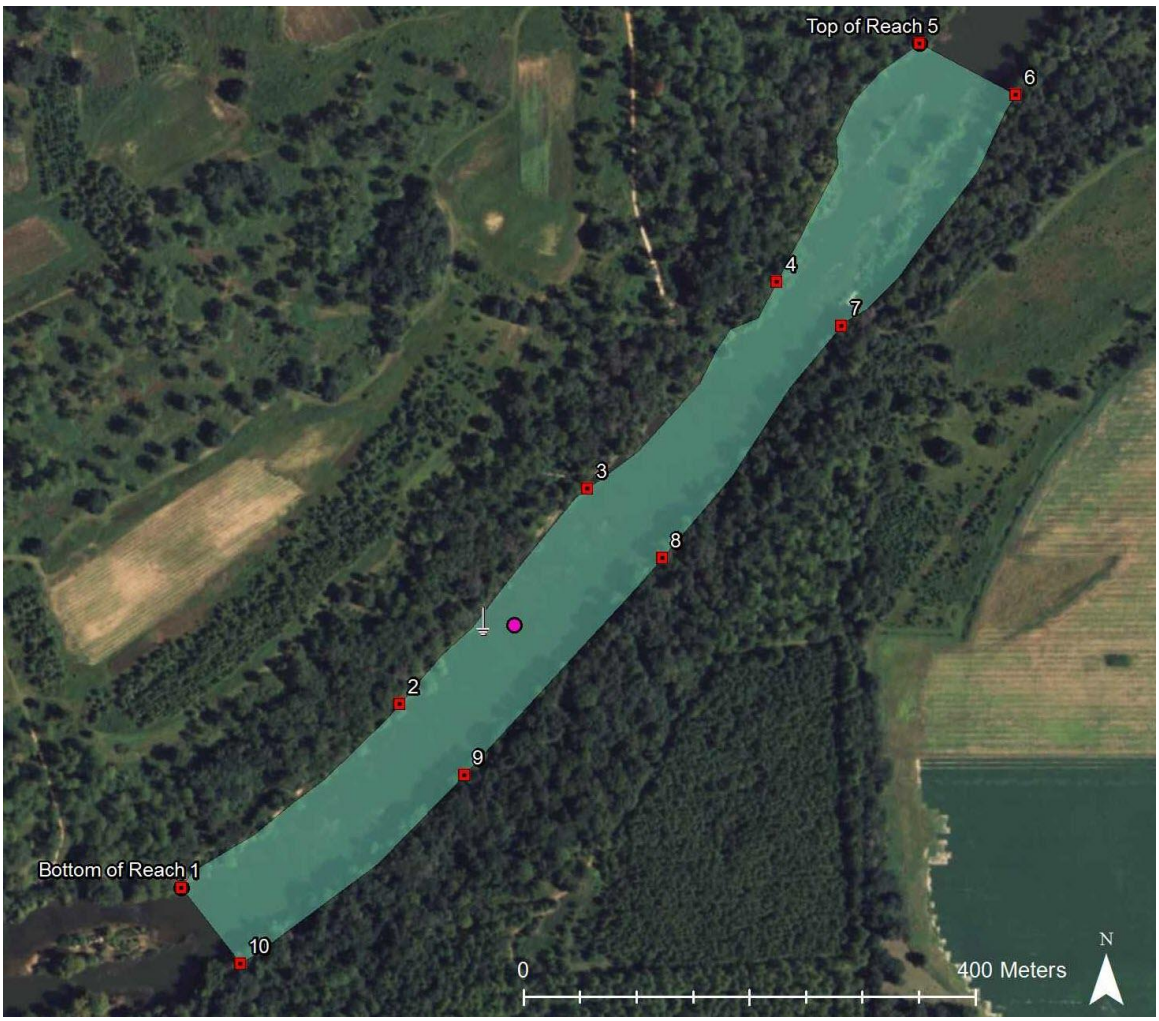


Figure 12. FLNT ideal riparian sampling design

7 REFERENCES

Keller, M., D. S. Schimel, W. W. Hargrove, and F. M. Hoffman. 2008. A continental strategy for the National Ecological Observatory Network. Frontiers in Ecology and the Environment. 6: 282-284.

Hargrove, W. W. and J. Pickering. 1992. Pseudoreplication: a sine qua non for regional ecology. Landscape Ecology 6: 251-258.

Hargrove, W. W., and F. M. Hoffman. 1999. Using multivariate clustering to characterize ecoregion borders. Computers in Science and Engineering. 1: 18-25.

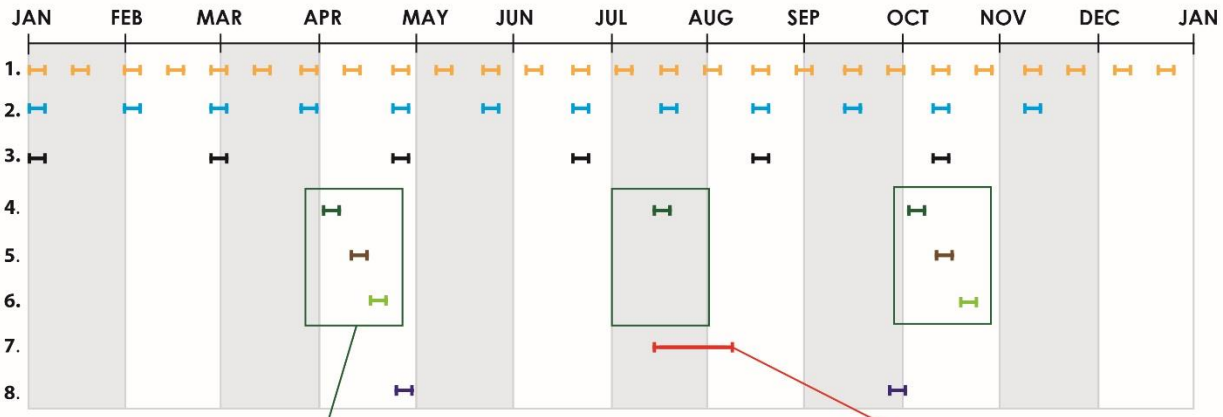
Hargrove, W. W., and F. M. Hoffman. 2004. Potential of Multivariate Quantitative Methods for Delineation and Visualization of Ecoregions. Environmental Management, 34: S39–S60.

Steele, J. H. 1978. Some comments on plankton patches. *In* Spatial pattern in plankton communities. *Edited by* J.H. Steele. Plenum Press, New York. pp. 1–20.

APPENDIX A LAKES

Note: Fish are not sampled in D03 lakes.

LAKE TEMPORAL SAMPLING STRATEGY



Bio Bout
4a. Aquatic Plant Clip Harvest (Bout 2)
4b. Aquatic Plant Point Pres/Abs (Bouts 1 & 3)
4c. Macroinvertebrates
4d. Periphyton, Phytoplankton
4e. Zooplankton
5. Sediment Chemistry (Bouts 1 & 3)
6. Fish (Bouts 1 & 3)

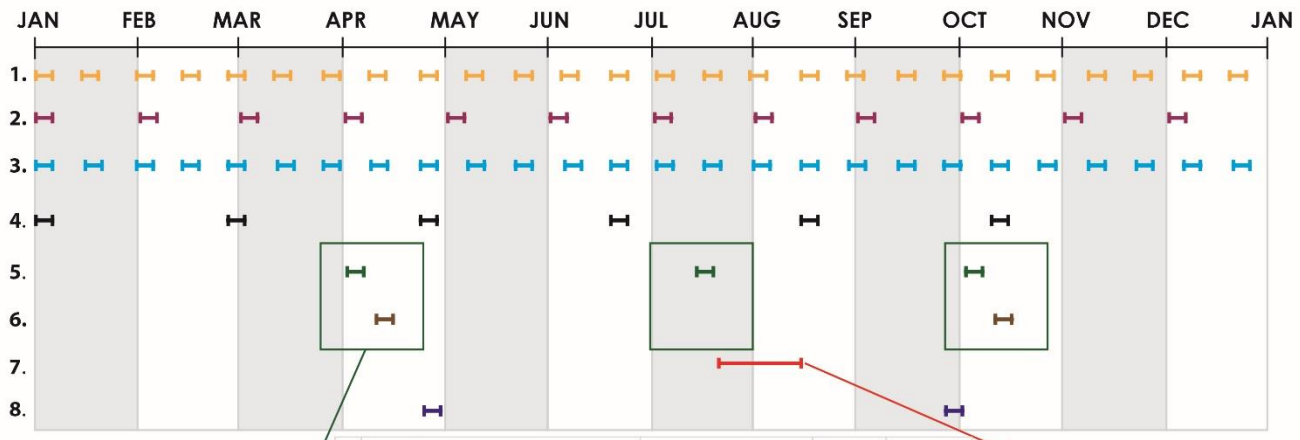
	SAMPLING BOUT	BOUTS/YEAR	FIELD HRS	LAB HRS
1	Sensor Maintenance	26	1 to 2	0
2	Water Chemistry, Isotopes, Dissolved Gas	12	1 to 3	1 to 3
3	Surface Microbes	12	2 to 4	0 to 1
4	Biology			
	a. Aquatic Plant Clip Harvest	1	3 to 8	4 to 8, 4, 2*
	b. Aquatic Plant Point Pres/Abs	2	3 to 8	0
	c. Macroinvertebrates	3	4	1 to 2
	d. Periphyton, Phytoplankton	3	3	4 to 8, 2, 2*
	e. Zooplankton	3	4	1 to 2
5	Sediment Chemistry	2	4 to 8	1 to 4
6	Fish	2	8 to 40*	1 to 4
7	Peak Greenness			
	a. Riparian Habitat Assessment	1	4 to 8	0
	b. Bathymetry	<1	8 to 40*	0
8	Groundwater Chemistry	2	6 to 20*	3 to 8

* indicates completion over multiple days

Peak Greeness
7a. Riparian Habitat Assessment
7b. Bathymetry (every 5 yrs)

APPENDIX B RIVERS

RIVER TEMPORAL SAMPLING STRATEGY



Bio Bout
5a. Aquatic Plants Clip Harvest (Bout 2)
5b. Aquatic Plants Pres/Abs (Bouts 1 & 3)
5c. Macroinvertebrates
5d. Periphyton, Phytoplankton
5. Sediment Chemistry (Bouts 1 & 3)

Peak Greenness
7a. Riparian Habitat Assessment
7b. Bathymetry (every 5 yrs)

	SAMPLING BOUT	BOUTS/YEAR	FIELD HR	LAB HRS
1	Sensor Maintenance	26	1 to 2	0
2	River discharge	12	1 to 3	0
3	Water Chem, Isotopes, Dissolved gas	26	1 to 3	1 to 3
4	Surface Microbes	12	2 to 4	0 to 1
5	Biology			
	a. Aquatic Plants Clip Harvest	1	3 to 8	2 to 8, 4, 2*
	b. Aquatic Plants Point Pres/Abs	2	3 to 8	0
	c. Macroinvertebrates	3	4	1 to 2
	d. Periphyton, Phytoplankton	3	3	3 to 8, 2, 2*
6	Sediment	2	4 to 8	1 to 4
7	Peak Greenness			
	a. Riparian Habitat Assessment	1	4 to 8	0
	b. Bathymetry	1	8 to 40*	0
8	Groundwater Chemistry	2	6 to 20*	3 to 8

* indicates completion over multiple days

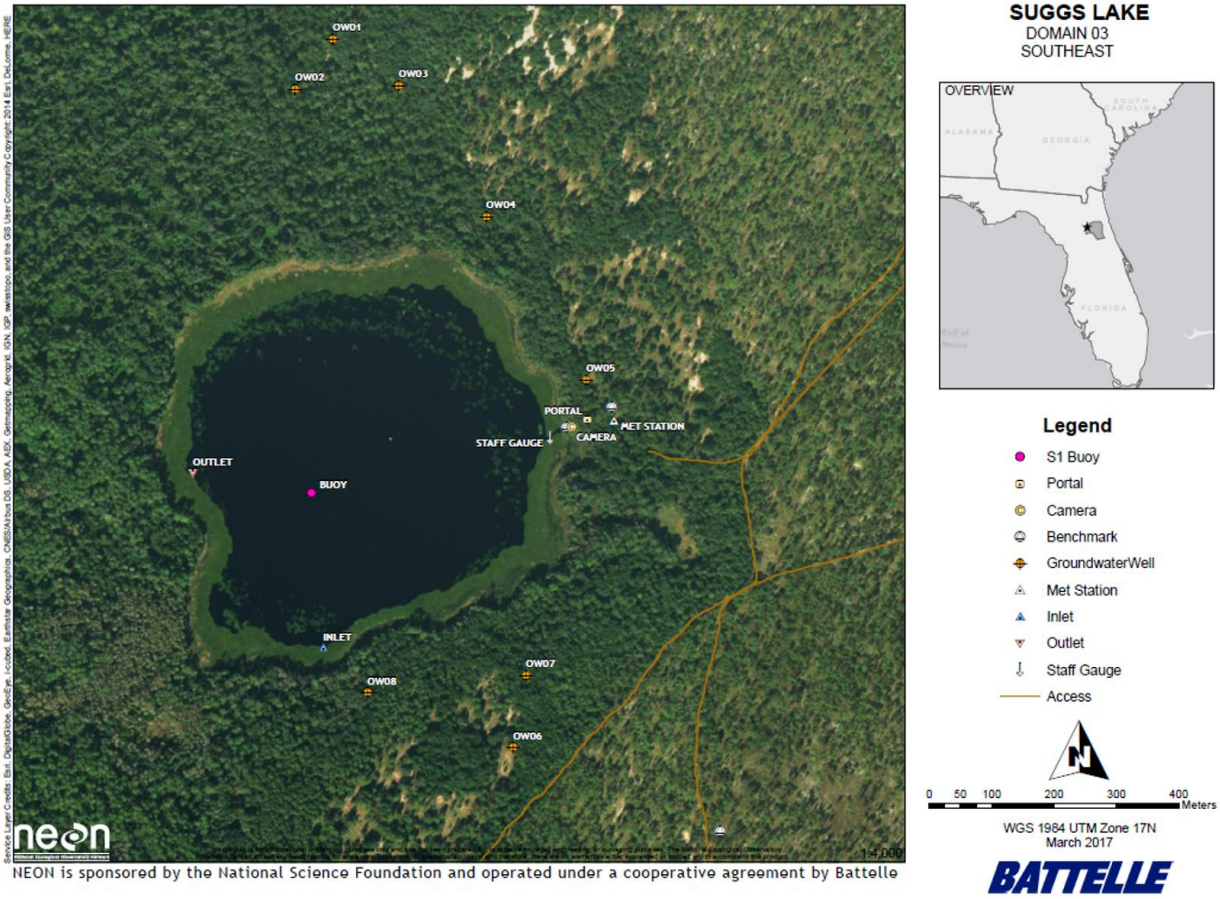
APPENDIX C OBSOLETE LOCATIONS, SUGG

We initially collected water chemistry samples at lake inlet, outlet, and buoy locations. After an analysis of data and discussions with external community members, we concluded that the water chemistry in lakes without true inlets and outlets were not significantly different at inlet and outlet locations relative to the buoy. Thus, to optimize OS sampling funds, we have removed lake inlet and outlet water chemistry sampling at lakes without true inlets and outlets and will only sample water chemistry at the lake buoy location.

Groundwater chemistry samples were initially collected at wells widely distributed around the site, and have since narrowed the strategy to sample two wells on the inlet side and two on the outlet side of the lake.

Location ID	***Description***	***Proposed Latitude***	***Proposed Longitude***	***Field Sci Latitude***	***Field Sci longitude***
Inlet	Inlet location from SCR	29.685553	-82.019685		
Outlet	Outlet location from SCR	29.690379	-82.017599		

* Riparian coordinates should be approximately evenly spaced throughout the sampling area



NEON is sponsored by the National Science Foundation and operated under a cooperative agreement by Battelle

APPENDIX D OBSOLETE LOCATIONS, FLNT

FLNT Obsolete Locations		
Station	Latitude	Longitude
1	31.188501°	-84.435308°
2	31.187729°	-84.435644°
3	31.186998°	-84.436064°
4	31.186439°	-84.436750°
5	31.185868°	-84.437430°
6	31.185326°	-84.438125°
7	31.184707°	-84.438763°
8	31.184097°	-84.439338°
9	31.183555°	-84.440053°
10	31.183052°	-84.440762°
S1	na	na
S2	na	na
Discharge	na	na
TOP of Reach	31.189113°	-84.434546°
BOT of Reach	31.182527°	-84.441632°