Aquatic Site Sampling Design – NEON Domain 09

PREPARED BY	**ORGANIZATION**	**DATE**
Stephanie Parker	AQU	5/7/2019
Nora Catolico	AQU	1/24/2018
Keli Goodman	AQU	2/7/2014
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 groundwater sampling wells for PRLA; updated PRPO and PRLA maps; Updated bathymetry timing; Updated bio contingencies and water and sediment chem sampling locations in seepage lakes
A	05/23/2019	ECO-06095	Initial Release

TABLE OF CONTENTS

1 DESCRIPTION 1
1.1 Purpose 1
1.2 Scope 1
2 RELATED DOCUMENTS AND ACRONYMS 1
2.1 Applicable Documents 1
2.2 Reference Documents 2
2.3 Acronyms 2
3 TEMPORAL SAMPLING STRATEGY 3
3.1 Rationale 3
3.2 Approach 3
4 SAMPLING DATES 6
4.1 Sensor Maintenance 6
4.2 Water Chemistry Sampling Dates 6
4.3 Groundwater Chemistry Sampling Dates 7
4.4 Biology Bout, Sediment Chemistry Sampling, and Riparian Assessment Dates 8
4.4.1 PRPO and PRLA Suggested Biology and Sediment Chemistry Bout 10
4.4.2 Other Biology Sampling 10
5 PROTOCOL DISTURBANCE AND PRIORITIZATION 12
5.1 Disturbance Criteria 12
6 SPATIAL SAMPLING STRATEGY 12
6.1 General Site Sampling Locations 12
6.2 Site Access and Instrument Locations 16
6.3 Riparian Sampling Locations 18
7 REFERENCES 20
APPENDIX A LAKES 21
APPENDIX B OBSOLETE LOCATIONS, PRPO 22
APPENDIX C OBSOLETE LOCATIONS, PRLA 23

LIST OF TABLES AND FIGURES

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.)............. 4
Table 2. Rule sets for sampling modules in lakes. Deviations may be allowed with science approval. 5
Table 3. Proposed surface water chemistry sampling dates for D09 Prairie Pothole and Prairie Lake. Dates are estimated based on available local data and may shift based on actual site conditions. Please note that dates are suggested, but should be adjusted as necessary, following the guidelines above. Although Tuesdays are the target, sampling may be shifted so that water chemistry sampling at one site occurs on Mondays while sampling at the second site occurs on Tuesdays, for example......................... 7
Table 4. Groundwater Observation Wells at D09 Prairie Pothole and Prairie Lake. Wells for groundwater chemistry sampling are denoted in bold text. 8
Table 5. Proposed groundwater chemistry sampling dates for D09 Prairie Pothole and Prairie Lake. 8
Table 6. Proposed Biological sampling windows in D09 Prairie Pothole and Prairie Lake. Sediment chemistry and fish sampling will take place during Bouts 1 and 3. The riparian habitat assessment peak greenness window may not coincide with the bout windows. 9
Table 7. Disturbance Criteria for lake 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. 12
Table 8. Module-specific sampling locations. 13
Table 9. PRPO 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. 15
Table 10. PRLA 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. 15

Figure 1. Proposed bouts for biological sampling at Prairie Pothole. Sediment chemistry and fish sampling occur during Bouts 1 and 3.. 11
Figure 2. Proposed bouts for biological sampling at Prairie Lake. Sediment chemistry and fish sampling occur during Bouts 1 and 3. 11
Figure 3. General diagram for an AQU site showing sampling locations in a seepage lake system........... 14
Figure 4. Site access and instrument locations at D09 Prairie Pothole. 16
Figure 5. Site access and instrument locations at D09 Prairie Lake.. 17
Figure 6. PRPO ideal riparian sampling design 18
Figure 7. PRLA ideal riparian sampling design 19

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 09 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	Center or 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

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. The general layout of a NEON lake site are presented in Section 6.

3.2 Approach

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

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).

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 2) 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.)

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
Fish	8-40	2	Ice-off Wind	Precipitation Water Temperature	Medium
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. Rule sets for sampling modules in lakes. 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 ^{o}C freezer. If processing in the domain lab, freeze at -80 ^{o}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 ^{o}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.
Fish	Schedule within 2 weeks of macroinvertebrate collection (biology bouts 1 and 3). Contingency situations may cause this time to be greater than 2 weeks.
	If conditions do not allow for fish sampling during bout 1, then sample when safe conditions allow up to 2 weeks before the start of bout 2. If conditions do not allow for fish sampling to occur during bout 3, then sample when safe conditions allow up to 30 days beyond the end of bout 3.
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.

4 SAMPLING DATES

The surface water sampling strategy for the D09 lake sites (Prairie Pothole and Prairie Lake) 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-covered throughout parts of the year, surface water samples can be taken on a semi-monthly basis combined with more intensive sampling around ice-on and ice-off, while organismal sampling is based on accumulation of growing degree days throughout the season.

The following Tables and Figures indicate proposed sampling dates for all sample protocols to be undertaken at Domain 09 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 buoys (RD[17]).

4.2 Water Chemistry Sampling Dates

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. If you cannot sample all sites on the same day, prioritize the core site for Tuesday sampling and sample the other site the following day or, if necessary, the following Tuesday.

Because these sites experience sustained winter temperatures below 0 °C, sampling will be less frequent than once monthly during the winter months and more frequently than once a month around the shoulder periods when turnover occurs in the lake coinciding with ice-on and ice-off dates (Table 3). Ice-off can be evaluated remotely by monitoring the staff gauge camera feed at the domain support facility. Ice-off in lakes is defined by the first loss of ice from the center of the lake in the spring. Ice-on in lakes is defined by the first ice coverage of the central part of the lake in the fall. Stratification can be determined by remotely evaluating temperature chain data.

Ice-off sampling strategy: One sample bout should occur one month prior to the long-term average of ice off conditions. The following sampling should occur within 1 week (maximum 2) of ice-off conditions assuming safe conditions allow access to the water body.

Ice-on sampling strategy: One sample should occur 2 weeks prior to the long-term ice-on averages for the region. Safe conditions for access to the lake must be met. The following sampling bout should occur 2 months after long-term ice-on averages for the region. Safe conditions require a minimum of 6" of ice to be able to safely access the lake for sampling.

Table 3. Proposed surface water chemistry sampling dates for D09 Prairie Pothole and Prairie Lake. Dates are estimated based on available local data and may shift based on actual site conditions. Please note that dates are suggested, but should be adjusted as necessary, following the guidelines above. Although Tuesdays are the target, sampling may be shifted so that water chemistry sampling at one site occurs on Mondays while sampling at the second site occurs on Tuesdays, for example.

PRPO/PRLA Bout	Date
1	First Tuesday of January (assuming safe conditions)
2	First Tuesday of February (assuming safe conditions)
3	Last Tuesday of estimated safe ice on (usually between March 1st and April 1st) (assuming safe conditions)
4	First Tuesday after ice off (usually between April 1st and May 15th)(assuming safe conditions)
5	First Tuesday of May
6	First Tuesday of June
7	First Tuesday of July
8	First Tuesday of August
9	First Tuesday of September
10	Third Tuesday of October (assuming safe conditions)
11	Last Tuesday of anticipated ice off (usually between Oct. 15th and Dec. 1st) (assuming safe conditions)
12	First Tuesday of estimated safe ice on (usually between Nov. 15th and Dec. 15th)(assuming safe conditions)

4.3 Groundwater Chemistry Sampling Dates

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 4). 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 5, has been selected to target one sampling event in the spring and one in the autumn conditions. Groundwater chemistry should be sampled on or near the target date if at all possible. 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. 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 4. Groundwater Observation Wells at D09 Prairie Pothole and Prairie Lake. **Wells for groundwater chemistry sampling are denoted in bold text.**

PRPO Well ID	Latitude	Longitude
D09-PRPO-OW-01	**47.129122**	**-99.251586**
D09-PRPO-OW-02	47.127513	-99.253772
D09-PRPO-OW-03	**47.127313**	**-99.255077**
D09-PRPO-OW-04	47.12787	-99.255362
D09-PRPO-OW-05	**47.129696**	**-99.255149**
D09-PRPO-OW-06	47.131507	-99.251965
D09-PRPO-OW-07	**47.131703**	**-99.250605**
D09-PRPO-OW-08	47.130911	-99.250276
PRLA Well ID	**Latitude**	**Longitude**
D09-PRLA-OW-01	**47.1617294**	**-99.1186272**
D09-PRLA-OW-02	47.1617294	-99.1175239
D09-PRLA-OW-03	**47.1615070**	**-99.1185978**
D09-PRLA-OW-04	47.1600037	-99.1136070
D09-PRLA-OW-05	47.1600424	-99.1132021
D09-PRLA-OW-06	47.1588970	-99.1135572
D09-PRLA-OW-07	**47.1579177**	**-99.1139129**
D09-PRLA-OW-08	**47.1584312**	**-99.1148070**

Table 5. Proposed groundwater chemistry sampling dates for D09 Prairie Pothole and Prairie Lake.

PRPO, PRLA Well Bout	Start Dates	Target Date	End Dates
1	April 15	April 28	May 15
2	October 15	October 27	November 15

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

The biology bout windows for lakes are based on a combination of parameters at each site. Using 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 1 and Figure 2), 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 6).

- Surface water microbes in lakes should be sampled in conjunction with the standard recurrent water chemistry sampling, every other month, 6 times per year. If water chemistry sample timing fluctuates due to ice on/ice off dates, sample on every other water chemistry sampling date.
  - 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, and fish) as well as sediment chemistry, follow pre-determined sampling windows presented in Table 6. Sediment chemistry and Fish are 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 6 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 and fish which may take up to 5 days at a site.
  - Bout 3 fish sampling may occur up to 30 days past the end of the Bout 3 window, if conditions allow (i.e., flowing water is present and it is safe to sample). Fish sampling should be scheduled within the bout windows, however sampling may be pushed later using this contingency as weather dictates.
- The riparian habitat assessment will occur within the dates provided in Table 6.

At northern lakes where ice on/ice off dates are a consideration, consider the suggested sampling bouts a guideline. Sample within the windows provided below if possible. On years where the ice off date is later than the start of Bout 1, adjust the window to 1 month after the date of actual ice off for sampling.

Table 6. Proposed Biological sampling windows in D09 Prairie Pothole and Prairie Lake. Sediment chemistry and fish sampling will take place during Bouts 1 and 3. The riparian habitat assessment peak greenness window may not coincide with the bout windows.

PRPO Bio Bout	Start Date	End Date
1	April 20	May 18
2	July 5	August 2
3	September 11	October 9
Riparian	June 8	August 20
PRLA Bio Bout	**Start Date**	**End Date**
1	April 18	May 16
2	July 5	August 2
3	September 11	October 9
Riparian	June 8	August 20

In D09, given the proximity of the lake sites, schedule sampling dates with decontamination of equipment in mind. One suggestion is to complete sampling at PRLA first, then PRPO the following week (or vice versa).

4.4.1 PRPO and PRLA Suggested Biology and Sediment Chemistry Bout

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)
3. Fish (Bouts 1 and 3 only)

4.4.2 Other Biology Sampling

- 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

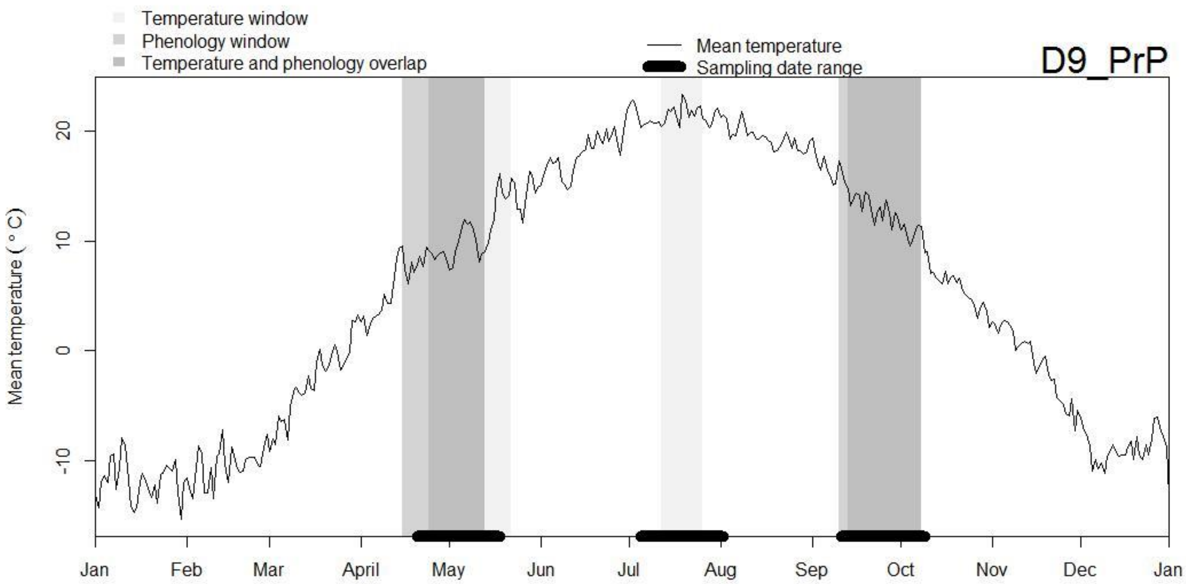


Figure 1. Proposed bouts for biological sampling at Prairie Pothole. Sediment chemistry and fish sampling occur during Bouts 1 and 3.

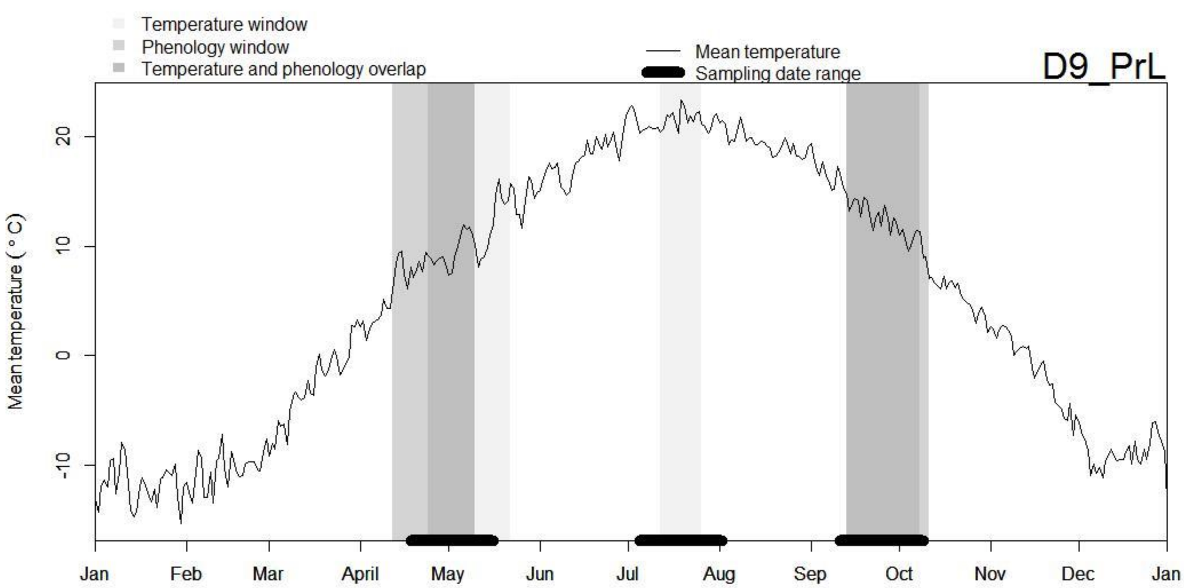


Figure 2. Proposed bouts for biological sampling at Prairie Lake. Sediment chemistry and fish sampling occur during Bouts 1 and 3.

5 PROTOCOL DISTURBANCE AND PRIORITIZATION

5.1 Disturbance Criteria

Each aquatic protocol has its own unique sensitivity to disturbance and perturbations (Table 7). These sensitivities should dictate the order in which protocols are completed.

Table 7. Disturbance Criteria for lake 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.

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
Periphyton and phytoplankton	6 hours- no disturbance that causes turbid conditions	2	Wading and substrate disturbance near shore, boat activity near sensor locations
Fish	6 hours- no disturbance that causes turbid conditions	4	Boat activity and wading nearshore.
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; Table 8). Lake sites are also divided into 10 sections divided by the riparian bank locations (Figure 3), which are used when sampling macroinvertebrates, periphyton, and the riparian habitat assessment. Locations provided in the "proposed" columns in Table 9 and Table 10, are estimates. Specific coordinates at each site will be used for the life of the site.

Table 8. Module-specific sampling locations.

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)
Fish	In riparian sections, exact location determined by field ecologists
Sediment chemistry	Buoy, lake inlet sensor (adjusted in-lake location for bio sampling)
Groundwater wells	Locations determined by HQ
Bathymetry	Whole lake

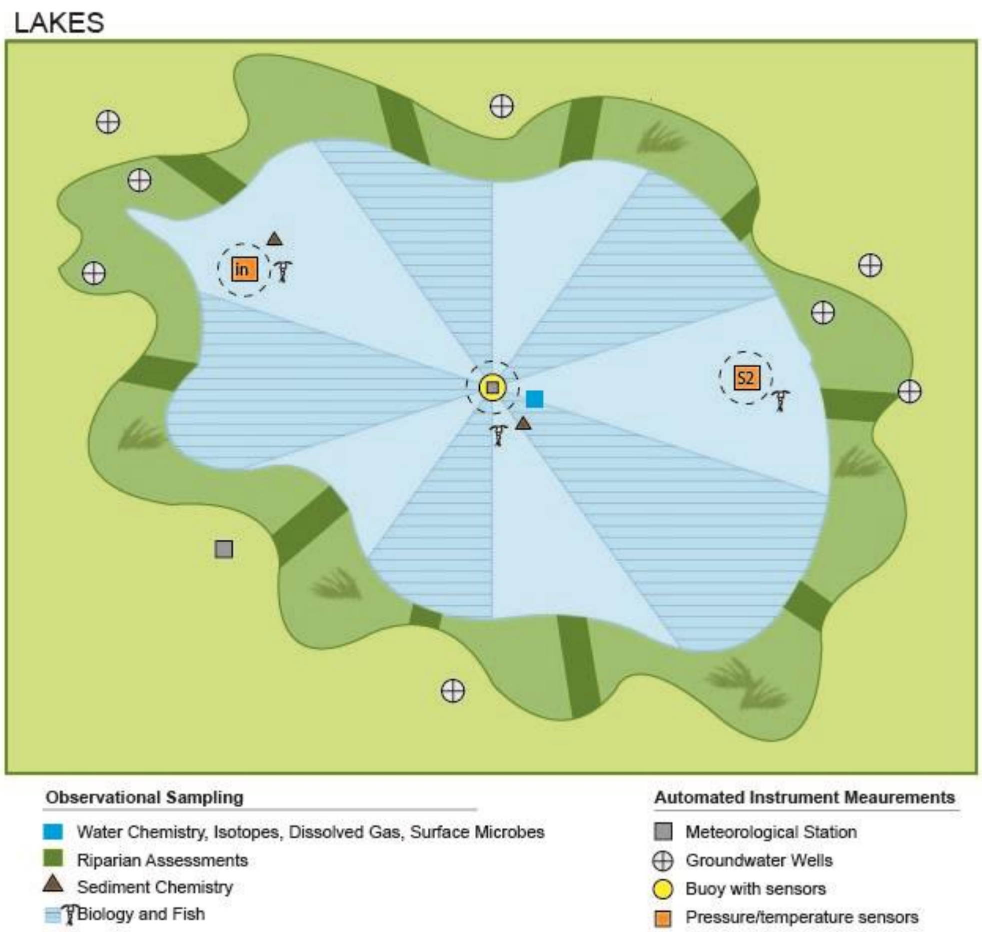


Figure 3. General diagram for an AQU site showing sampling locations in a seepage lake system.

Table 9. PRPO 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*	47.130346	-99.250416	47.130336	-99.250391
02 - Riparian	Riparian coordinates*	47.129317	-99.251553	47.129339	-99.251479
03 - Riparian	Riparian coordinates*	47.128432	-99.252664	47.128397	-99.252532
04 - Riparian	Riparian coordinates*	47.127649	-99.25389	47.127615	-99.253850
05 - Riparian	Riparian coordinates*	47.128022	-99.255104	47.127959	-99.255080
06 - Riparian	Riparian coordinates*	47.129147	-99.255118	47.129155	-99.255139
07 - Riparian	Riparian coordinates*	47.130206	-99.25475	47.130142	-99.254748
08 - Riparian	Riparian coordinates*	47.13138	-99.254489	47.131309	-99.254597
09 - Riparian	Riparian coordinates*	47.131687	-99.2527	47.131673	-99.252767
10 - Riparian	Riparian coordinates*	47.131377	-99.250997	47.131438	-99.251132
Inlet	Inlet location from SCR	47.158845	-99.114183	47.129671	-99.254694
Outlet	Outlet location from SCR	47.131028	-99.250619	47.131108	-99.250809
C0 (buoy)	S1 sensor location from SCR	47.130634	-99.253345		

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

Table 10. PRLA 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*	47.160380	-99.120881	47.160410	-99.120862
02 - Riparian	Riparian coordinates*	47.161490	-99.118052	47.161496	-99.118068
03 - Riparian	Riparian coordinates*	47.161693	-99.115780	47.161713	-99.115755
04 - Riparian	Riparian coordinates*	47.162795	-99.115030	47.162789	-99.115020
05 - Riparian	Riparian coordinates*	47.161958	-99.113439	47.161979	-99.113416
06 - Riparian	Riparian coordinates*	47.160542	-99.113856	47.160532	-99.113847
07 - Riparian	Riparian coordinates*	47.158548	-99.114723	47.158533	-99.114713
08 - Riparian	Riparian coordinates*	47.157119	-99.116611	47.157105	-99.116611
09 - Riparian	Riparian coordinates*	47.158057	-99.119214	47.158039	-99.119199
10 - Riparian	Riparian coordinates*	47.158407	-99.121175	47.158420	-99.121143
Inlet**	Inlet location used from 09/2018 to present	N/A	N/A	47.159104	-99.114273
Outlet	Outlet location from SCR	47.160880	-99.120252	47.160881	-99.120249
C0 (buoy)	S1 sensor location from SCR	47.159794	-99.118729		

* Riparian coordinates should be approximately evenly spaced throughout the sampling area
**Inlet location moved deeper due to drop in water levels causing sensors to be out of water

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 to minimize local disturbance over the life of the site at locations (e.g., sensors and boat launches) that are accessed frequently (Figure 4 and Figure 5).

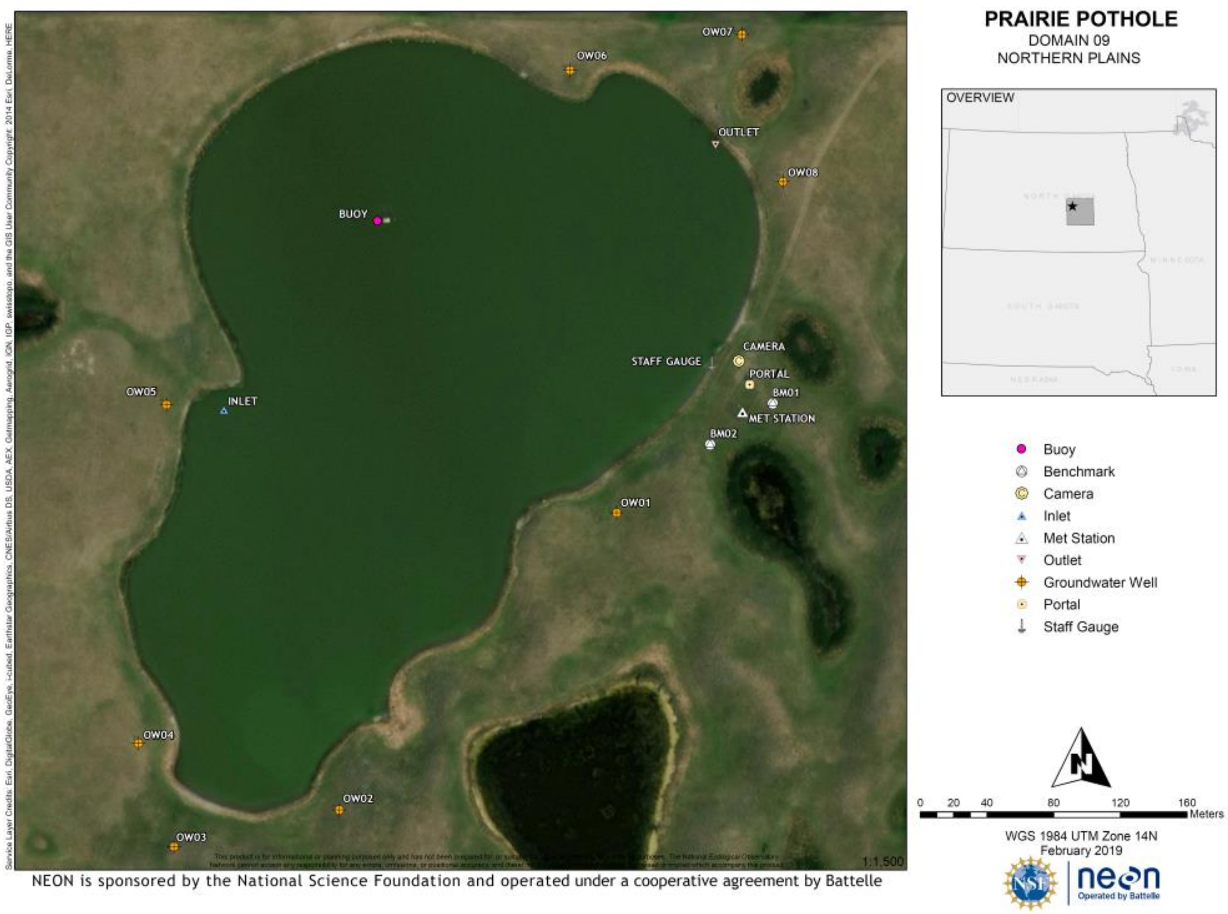


Figure 4. Site access and instrument locations at D09 Prairie Pothole.

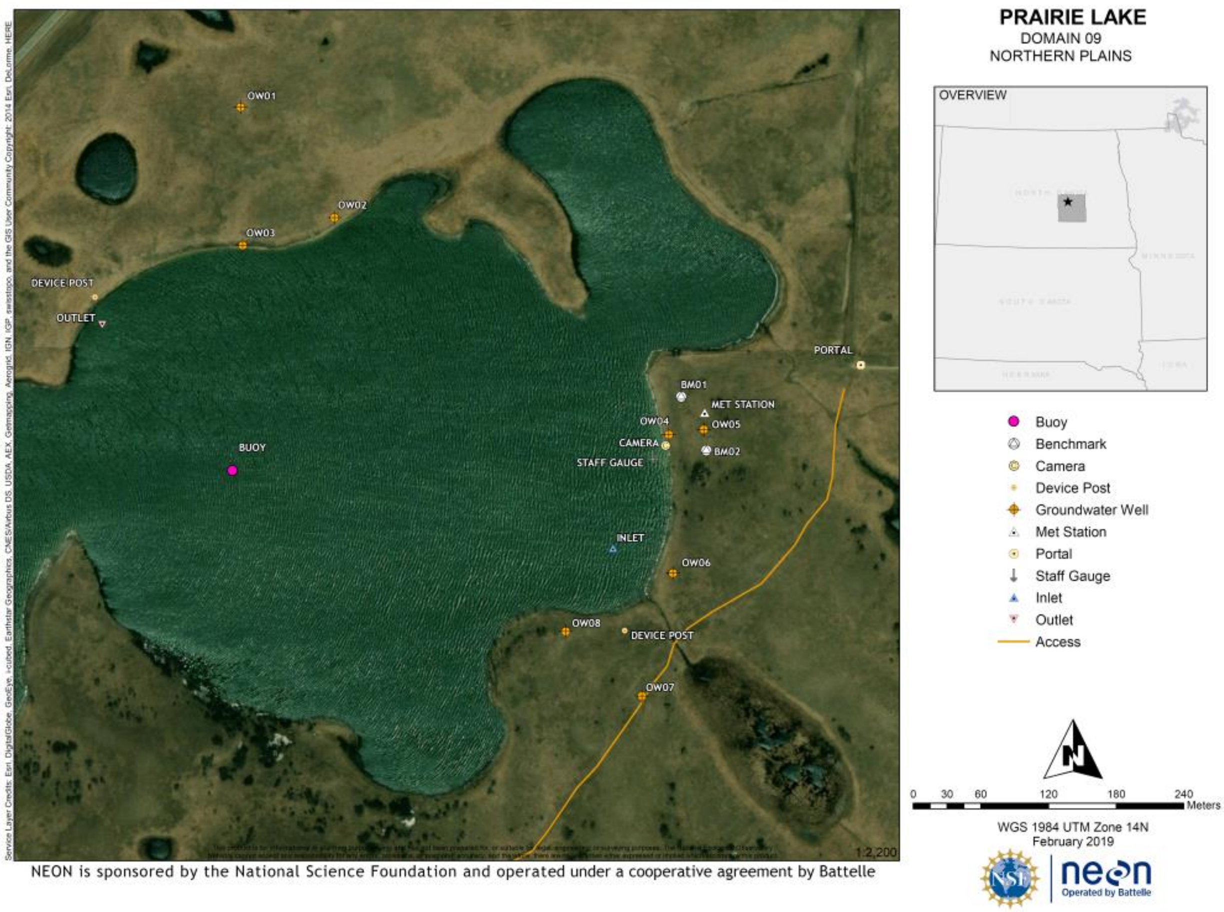


Figure 5. Site access and instrument locations at D09 Prairie Lake.

6.3 Riparian Sampling Locations

Riparian coordinates are determined prior to sampling at HQ. Coordinates at most sites are groundtruthed by Field Science in and reported back to Science to update Table 9 and Table 10. 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 6, Figure 7).

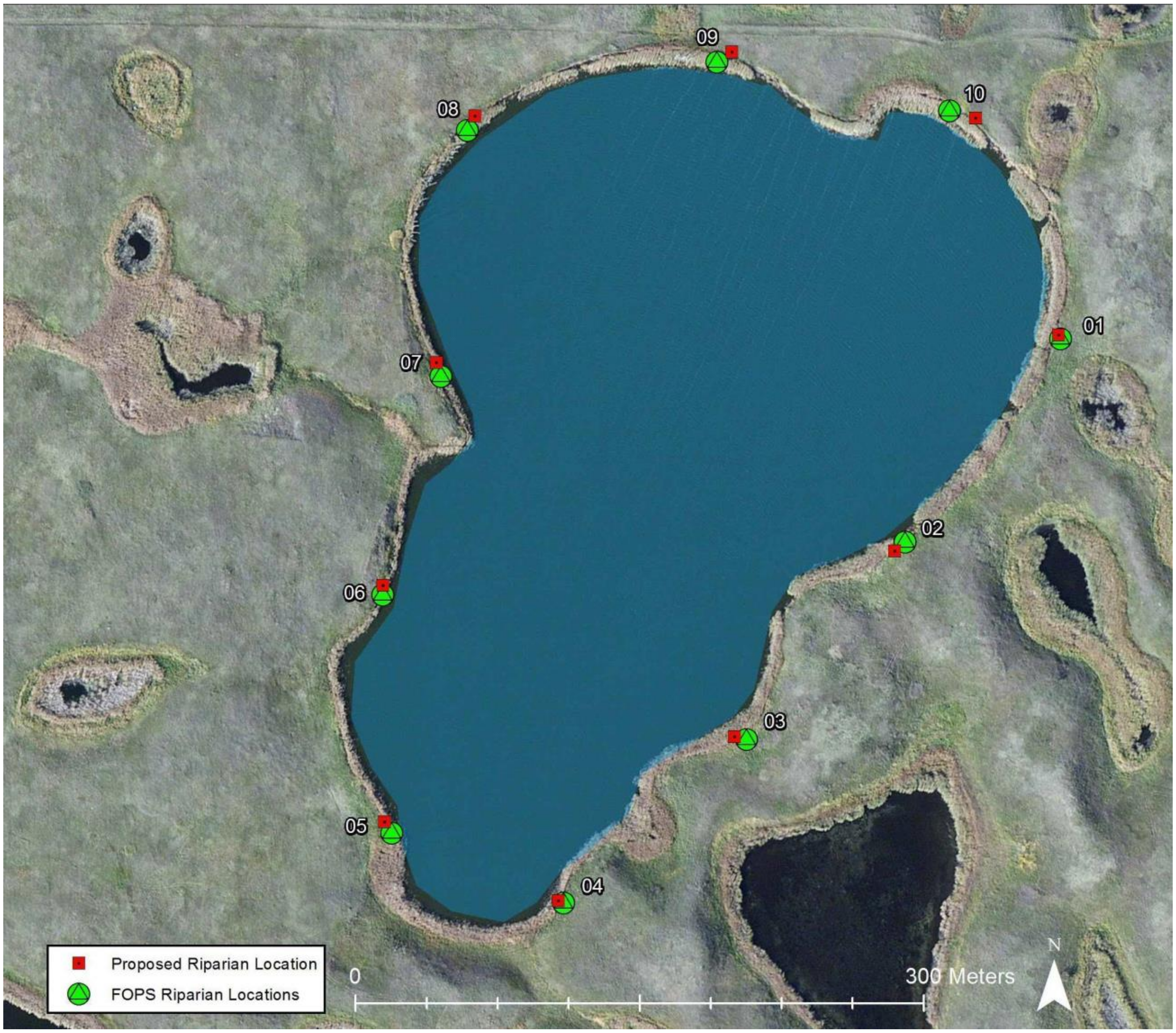


Figure 6. PRPO ideal riparian sampling design

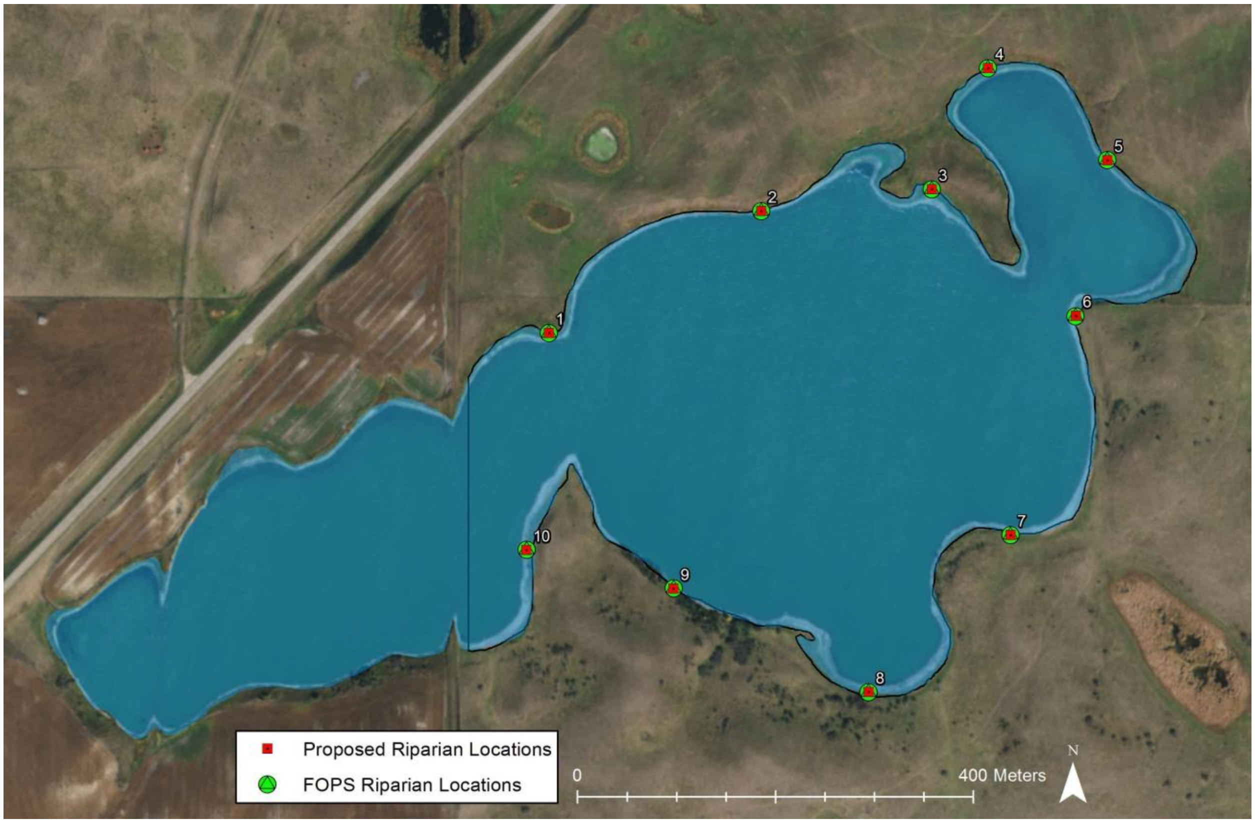


Figure 7. PRLA ideal riparian sampling design

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.

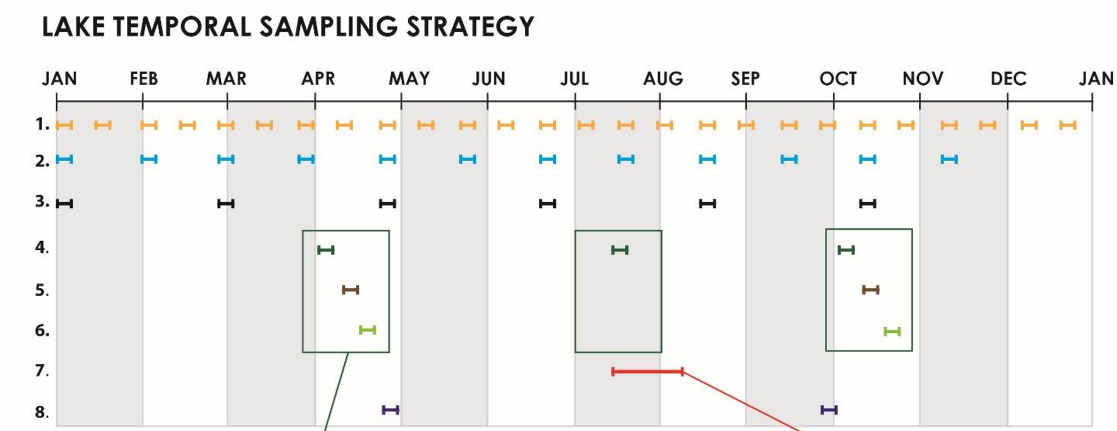
Hargrove, W. W. and J. Pickering. 1992. Pseudoreplication: a sine qua non for regional ecology. Landscape Ecology 6: 251-258.

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



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)

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

	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

Appendix B OBSOLETE LOCATIONS, PRPO

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.

PRPO Obsolete Locations		
Station	Latitude	Longitude
1	47.130346°	-99.250416°
2	47.129317°	-99.251553°
3	47.128432°	-99.252664°
4	47.127649°	-99.253890°
5	47.128022°	-99.255104°
6	47.129147°	-99.255118°
7	47.130206°	-99.254750°
8	47.131380°	-99.254489°
9	47.131687°	-99.252700°
10	47.131377°	-99.250997°
C0	47.130634	-99.253345
Inlet	47.12972	-99.254799

Appendix C OBSOLETE LOCATIONS, PRLA

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.

PRLA Obsolete Locations		
Station	Latitude	Longitude
1	47.158407°	-99.121175°
2	47.158057°	-99.119214°
3	47.157119°	-99.116611°
4	47.158548°	-99.114723°
5	47.160542°	-99.113856°
6	47.161958°	-99.113439°
7	47.162795°	-99.115030°
8	47.161693°	-99.115780°
9	47.161490°	-99.118052°
10	47.160380°	-99.120881°
C0	47.159794	-99.118729
Inlet	47.158853	-99.114188