Aquatic Site Sampling Design – NEON Domain 13

PREPARED BY	ORGANIZATION	DATE
Stephanie Parker	AQU	3/14/2019
Nora Catolico	AQU	1/24/2018
Keli Goodman	AQU	11/04/2016
Brandon Jensen	AQU	5/18/2018
Jenna Stewart	AQU	
Charlotte Roehm	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			Updated reaeration rules from 1 week of no disturbance prior to 2 days; Added rapid habitat assessment; CM updated with new template and changes based on riparian habitat assessment timing; Updated wording for reaeration timing; Updated Groundwater well sampling locations and site access map for COMO; Updated stream morphology timing and added frozen/snowy site wording; Updated WLOU site map and groundwater sampling locations; Updated bio contingencies. Sediment sampling frequency updates and revisions to fish sampling reach establishment. Updated formatting and content for several tables.
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 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.003162	AOS Protocol and Procedure: Wadeable Stream Morphology
RD [06]	NEON.DOC.000693	AOS Protocol and Procedure: Reaeration in Streams
RD [07]	NEON.DOC.002905	AOS Protocol and Procedure: Water Chemistry Sampling in Surface Waters and Groundwater
RD [08]	NEON.DOC.001886	AOS Protocol and Procedure: Stable Isotope Sampling in Surface and Ground Waters
RD [09]	NEON.DOC.001199	AOS Protocol and Procedure: Surface Water Dissolved Gas Sampling
RD [10]	NEON.DOC.001193	AOS Protocol and Procedure: Sediment Chemistry Sampling in Wadeable Streams
RD [11]	NEON.DOC.003044	AOS Protocol and Procedure: Aquatic Microbe Sampling
RD [12]	NEON.DOC.003045	AOS Protocol and Procedure: Periphyton, Seston, and Phytoplankton Sampling
RD [13]	NEON.DOC.003039	AOS Protocol and Procedure: Aquatic Plant, Bryophyte, Lichen, and Macroalgae Sampling
RD [14]	NEON.DOC.003046	AOS Protocol and Procedure: Aquatic Macroinvertebrate Sampling
RD [15]	NEON.DOC.003826	AOS Protocol and Procedure: Riparian Habitat Assessment
RD [16]	NEON.DOC.001295	AOS Protocol and Procedure: Fish Sampling in Wadeable Streams

2.3 Acronyms

AQU	Aquatic or reference reach
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
SDRC	Stage discharge rating curve
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 wadeable stream site is 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).

Physical/Chemical: Flow regime has been identified as the main variable defining the timing and frequency of sampling for the physical and chemical parameters.

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.

A large number of the sampling protocols are, in addition, constrained by other given variables. The overwhelming constraint for Aquatics is discharge related.

Flow Regime: The characteristic annual flow curves for a given stream.

Discharge: The volume of water flow through a given cross-sectional area per given unit of time.

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 wadeable streams 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 channel morphology.) Fish are not collected at D13 COMO due to permitting restrictions.

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					
Discharge	1-2	24	Flow	Flow Regime Precipitation	High
Reaeration	4-8	6	Flow	Flow Regime	High
Stream morphology	10-120	1 per 5 yrs*	Discharge Temperature	Flow Regime	Low to Medium
Rapid habitat assessment	4-8	1	Flow	Flow Regime	Medium
Biological					
Surface microbes	2-4	12	Discharge	Flow Regime Water Temperature	High
Aquatic Plants, Bryophytes, Lichens, and Macroalgae	3-8	3	Discharge	Flow Regime Light (PAR)	High
Macroinvertebrates	4	3	Discharge	Flow Regime Water Temperature	High
Periphyton and seston	3	3	Discharge	Flow Regime Light (PAR)	High
Benthic microbes	3	3	Discharge	Flow Regime Riparian greenness	High
Fish	8-40	2	Discharge	Flow Regime Water Temperature	Medium
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	6-20	2	Sufficient Water in Well	Flow Regime	Medium to High

Table 2. Rule sets for sampling modules in wadeable streams. Fish are not collected at D13 COMO due to permitting restrictions. 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.
	If the sensor location cannot be sampled due to drying, ice, etc., collect samples from an alternative location within the permitted reach and report the GPS coordinates and coordinate uncertainty in the field data.
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.
Reaeration	Sediments must not be majorly disturbed 1 hour prior to sampling within the sensor reach. Must complete discharge on days when reaeration is measured.
Aquatic plants, bryophytes, lichens, and macroalgae	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 or water returns to a dry channel, 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.
	Minimize biomass collection within sensor reach.
Macroinvertebrates	If a flood occurs or water returns to a dry channel, 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.
	Minimize sampling within sensor reach.
Periphyton, seston	If a flood occurs or water returns to a dry channel, wait a minimum of 14 days after the water level drops below 3x median discharge to allow the periphyton community to recolonize.
	Sample in conjunction with benthic microbes.
	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.
Benthic microbes	If a flood occurs or water returns to a dry channel, wait a minimum of 14 days after the water level drops below 3x median discharge to allow the benthic microbe community to recolonize.
	Sample in conjunction with periphyton.
	Samples 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.
Sediment chemistry	If a flood occurs or water returns to a dry channel, 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 biology sampling (but before fish) 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.
	In a contingency situation, fish sampling may occur before other biological sampling. A two week recolonization period must be observed after fish and before other biological sampling.

	If a flood occurs, wait a minimum of 3 days after the water level drops below 3x median discharge to allow the fish assemblage to redistribute. 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.
	Should be sampled after other biology modules because disturbance to stream bottom is high.
Stream morphology	Stream morphology should occur near baseflow conditions and when the riparian canopy is senesced.
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	Ensure that wading or other activities in the stream do not interfere with flow or bias discharge measurements during collection.
	A wide range of flow conditions should be targeted for measurement throughout the water year given safety considerations.
Rapid habitat assessment	Occurs between Biology/Sediment Chemistry Bouts 1 and 2, when conditions are safely wadeable and after riparian vegetation has leafed-out.

4 SAMPLING DATES

The surface water sampling strategies for Como Creek and West St. Louis Creek are based on hydrological data collected from a nearby, USGS hydrological monitoring location in larger watersheds. Thus, our sampling strategy may not accurately represent the hydrologic condition at the NEON site, and will need to be updated following annual data collections. Given the lack of long-term historical data at this site, Domain 13 staff needs to have the leeway to adjust sampling dates based on actual site conditions.

The following Tables and Figures indicate proposed sampling dates for all sample protocols to be undertaken at Domain 13 over the course of a year.

4.1 Sensor Maintenance

Sensor preventative maintenance for in-stream 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.

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.

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 (Table 3, Figure 1, Figure 2). 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 day (± 2 days) as a monthly Tuesday sample, adjust the flow-weighted sample by ± 7 days.

Account for missed sampling events during zero-flow periods by adding weekly water sample collections to the schedule once the flow has returned. Continue with weekly water samples until the number of samples missed is equal to the number of weekly samples collected. Total sample numbers should not exceed 26 times per year.

Table 3. Proposed stream water chemistry sampling dates for D13 Como Creek and West St. Louis Creek for the 14 samples collected to reflect the discharge related strategy.

COMO Proposed sampling date	WLOU Proposed sampling date
May 16	March 15
May 28	May 3
June 5	May 20
June 10	May 30
June 16	June 7
June 20	June 12
June 25	June 17
June 30	June 21
July 6	June 26
July 14	July 1
July 23	July 10
August 4	July 29
August 23	September 5
September 23	November 1

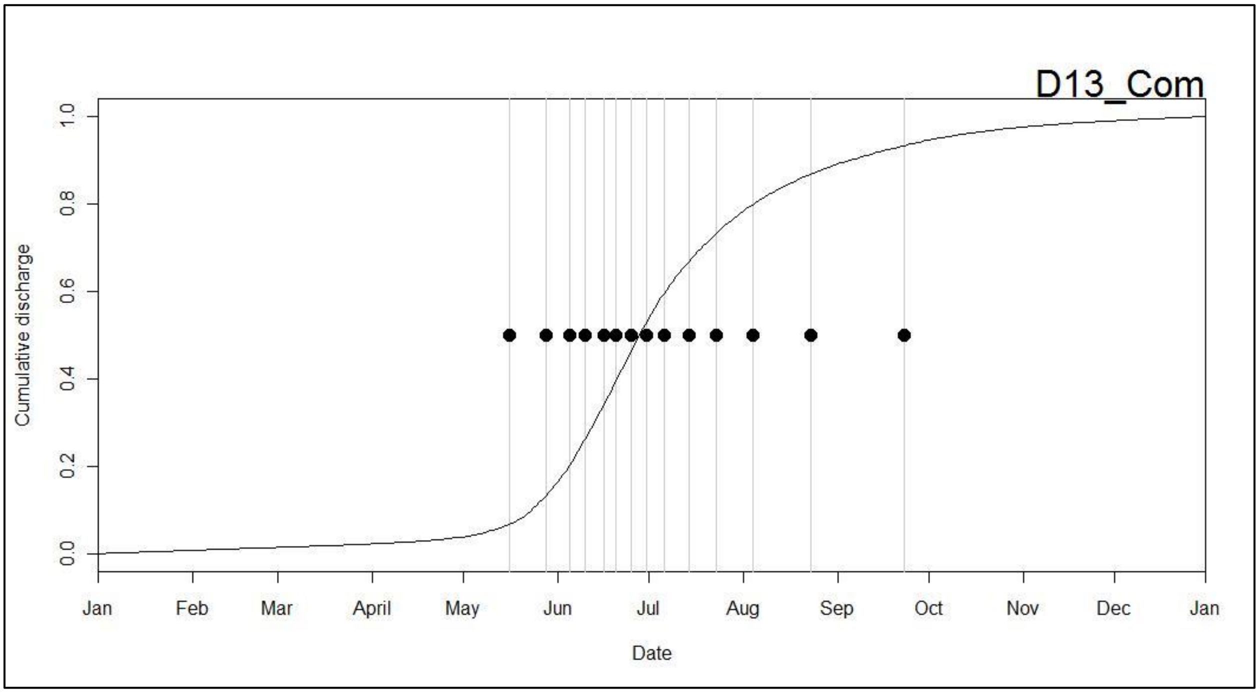


Figure 1. Timing of sample collection for 14 water chemistry samples reflecting the discharge related strategy for Como Creek.

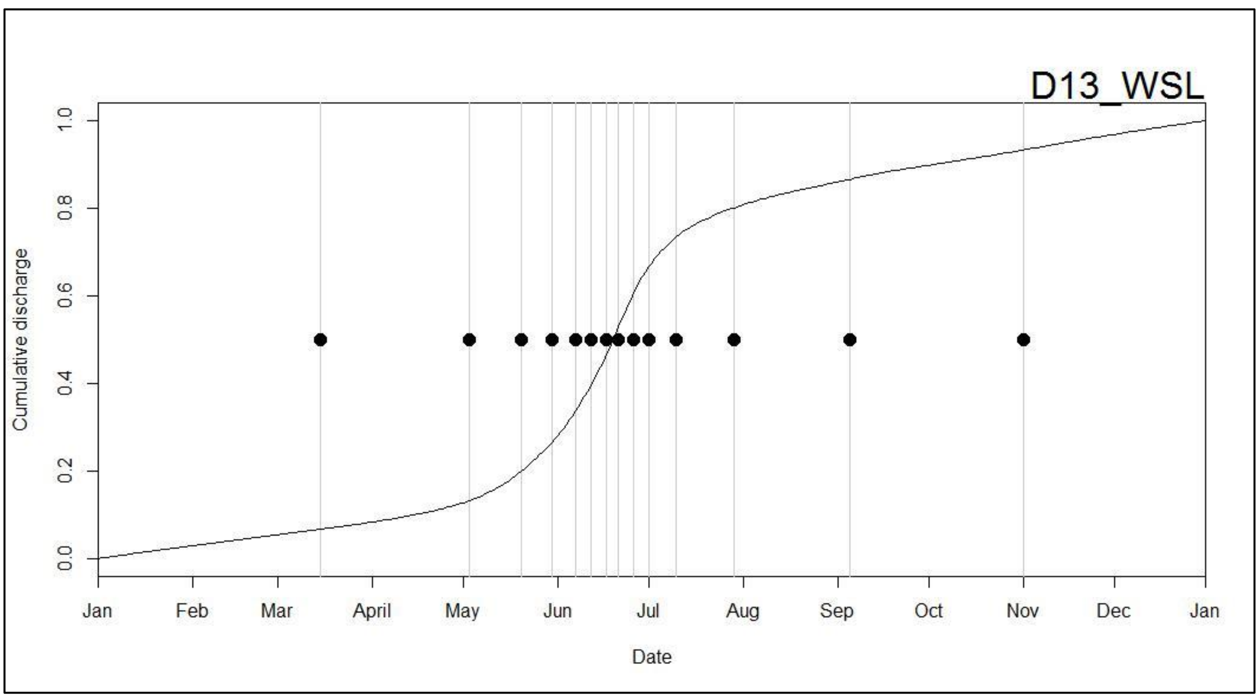


Figure 2. Timing of sample collection for 14 water chemistry samples reflecting the discharge related strategy for West St. Louis Creek.

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.

Groundwater samples will be collected twice per year from a subset of 4 wells per site (Table 4). The four sampling wells are selected in attempt to cover all of the following categories: upstream, downstream, right bank, and left bank. Preference is also given to wells that are closer to the surface water chemistry sampling locations. This strategy enables the study of surface-groundwater interaction in the hyporheic zone and 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 5 and shown in relation to the cumulative discharge in Figure 3 and Figure 4 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 4. Groundwater Observation Wells at D13 Como Creek and West St. Louis Creek. **Wells for sampling denoted in bold text.**

COMO Well ID	Latitude	Longitude
D13-COMO-OW-01	**40.035039**	**-105.544811**
D13-COMO-OW-02	**40.035081**	**-105.545205**
D13-COMO-OW-03	**40.034929**	**-105.544872**
D13-COMO-OW-04	**40.034756**	**-105.544637**
WLOU Well ID	**Latitude**	**Longitude**
D13-WLOU-OW-01	**39.891225**	**-105.914597**
D13-WLOU-OW-02	**39.891174**	**-105.914506**
D13-WLOU-OW-03	39.891283	-105.913598
D13-WLOU-OW-04	39.891052	-105.913134
D13-WLOU-OW-05	**39.891138**	**-105.912945**
D13-WLOU-OW-06	39.890995	-105.912219
D13-WLOU-OW-07	39.891026	-105.911437
D13-WLOU-OW-08	**39.890658**	**-105.911605**

Table 5. Proposed groundwater chemistry sampling dates for D13 Como Creek and West St. Louis Creek.

COMO Well Bout	Start Date	End Date
1	May 24	June 13
2	July 18	August 4
WLOU Well Bout	**Start Date**	**End Date**
1	April 24	May 18
2	August 10	September 12

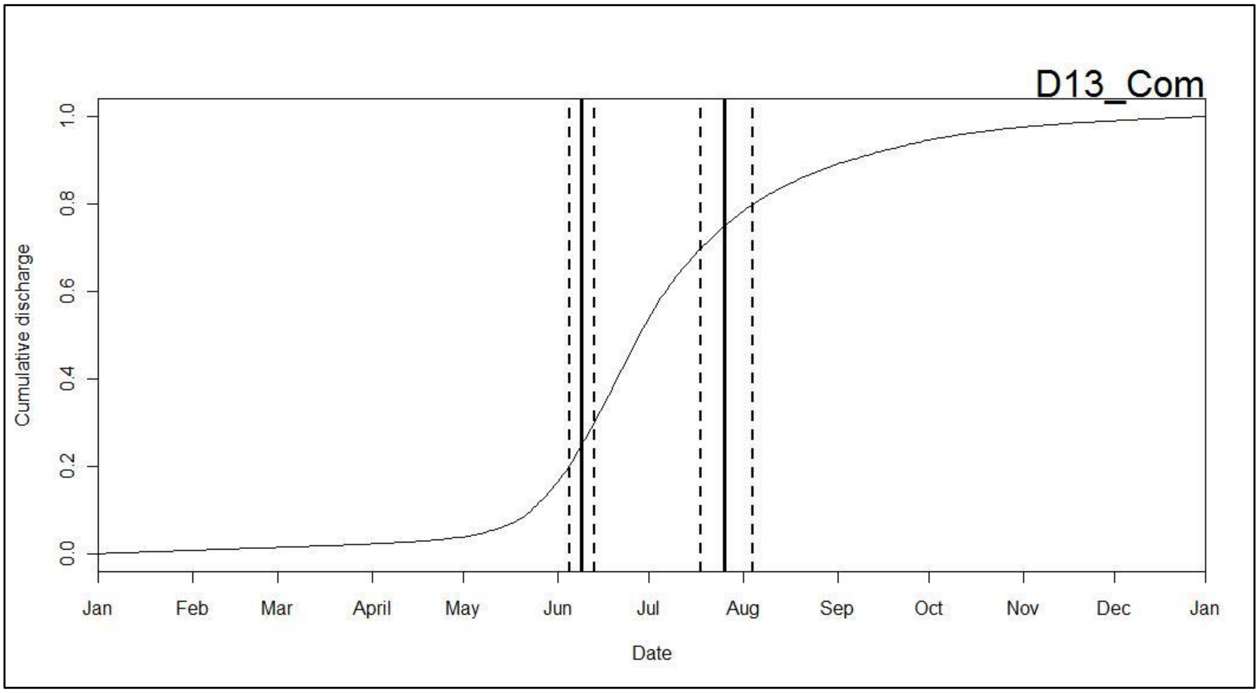


Figure 3. Timing of sample collection for 2 groundwater chemistry samples reflecting the discharge related strategy for Como Creek.

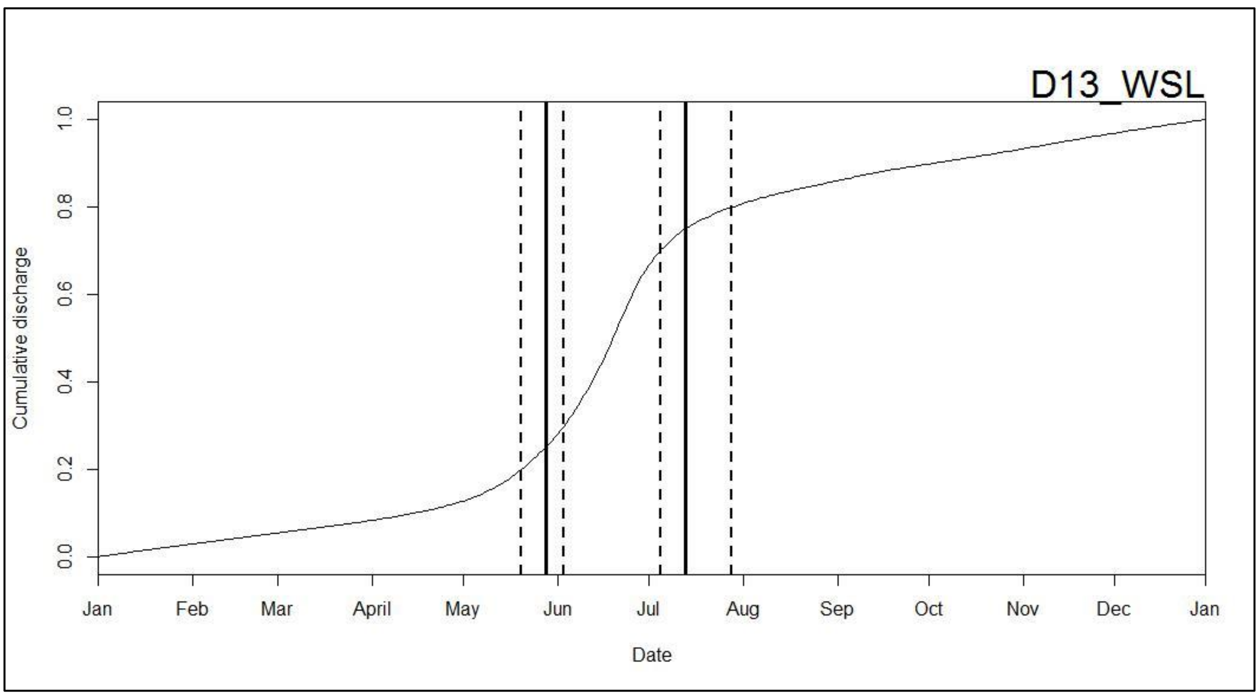


Figure 4. Timing of sample collection for 2 groundwater chemistry samples reflecting the discharge related strategy for West St. Louis Creek.

4.4 Discharge and Reaeration Sampling Dates

Stream discharge must be measured at aquatic sites with wadeable streams a minimum of twenty-four (24) times in order to establish a stage-discharge rating curve (SDRC) followed by twelve (12) times per year to validate and refine the rating. After the first year of sampling, the SDRC will be reviewed and approved by NEON Science. If the SDRC is not approved by Science, then Science will request that field operations staff schedule additional sampling bouts to fill in data gaps needed to improve the SDRC.

Discharge should be conducted when it is safe to wade in the stream. Once a discharge-rating curve is established, discharge conditions will be targeted to fill in underrepresented points along the rating curve. Discharge sampling requirements will represent annual flow conditions and be constrained by zero-flow days.

Reaeration measurements should initially be completed 10x/year and then 6x/year thereafter, in conjunction with discharge measurements. Sites that are high risk sites for flooding resulting in changes in stream morphology may be requested to continue to collect reaeration 10x/year. Sampling events should be spread out throughout the year so as to collect a range of flows. Reaeration measurements may be completed more frequently (up to 10x/year) if major changes in stream flow alter reaeration parameters and a new curve must be established. Discharge must be completed on the same day that reaeration is conducted.

Do not schedule discharge or reaeration during time periods when the stream is known to freeze over or freeze solid. If reaeration and discharge are scheduled and the stream is frozen, the sampling event(s) will need to be moved in the schedule.

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

The biology bout windows for wadeable streams are based on a combination of parameters at each site. Using USGS streamflow data from nearby "proxy" sites, mean daily air temperature (NOAA NCDC [NCEI] 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 5 and Figure 6), 1 month sampling windows were pre-determined for all sites. Sampling windows may be adjusted with stream flow as all biological sampling (except for surface water microbes) is based on actual conditions at the site. Biology Bout 1 is especially susceptible to changes at sites dominated by snowmelt in the spring. Bout dates may be adjusted after consultation with NEON science at sites affected by snowmelt. 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 wadeable streams should be sampled in conjunction with the standard recurrent water chemistry sampling, once per month, 12 times per year.

  - Surface water DNA microbe samples collected during July should be marked for metagenomics analysis.
- Sampling for all other biological modules (aquatic plants/macroalgae, macroinvertebrates, seston/periphyton) 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 fish which may take up to 5 days at a site.
  - Benthic microbe samples collected during Bout 2 should be marked for metagenomics analysis.
  - If weather or flooding dictates changes to the sampling schedule, the following contingencies may be applied:
    - Periphyton/phytoplankton, benthic microbes, aquatic plants, macroinvertebrates, and sediment chemistry sampling may be pushed later following flooding criteria in Table 2.
    - Sediment chemistry may be moved ahead of periphyton sampling as long as a 14 day re-colonization period is allowed prior to periphyton collection.
    - All biology/sediment chemistry and Bout 1 fish sampling may occur up to 2 weeks prior to the start date of the next sampling bout.
    - 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.
- The rapid habitat assessment (an SOP in the Wadeable Stream Morphology protocol) should take place between Bouts 1 and 2 to allow for water to resume to near-baseflow conditions and allow riparian areas to green-up.
  - If a stream morphology survey has recently occurred (within the same year), contact science to confirm that the rapid habitat survey is necessary.

Table 6. Proposed Biological and Sediment Chemistry sampling windows for D13 Como Creek and West St. Louis Creek. Sediment Chemistry will take place during Bouts 1 and 3. Fish sampling will take place during Bouts 1 and 3 at WLOU only. The riparian habitat assessment peak greenness window may not coincide with the bout windows.

COMO Bio Bout	Start Date	End Date
1	May 20	June 17
2	July 14	August 11
3	August 30	September 27

Riparian	June 30	August 31
WLOU Bio Bout	**Start Date**	**End Date**
1	May 2	May 30
2	July 5	August 2
3	September 3	October 1
Riparian	June 26	September 16

4.5.1 Suggested Biology and Sediment Chemistry Bout:

1. Aquatic plants, macroinvertebrates, periphyton/benthic microbes (in any order)
2. Sediment chemistry (Bouts 1 and 3 only)
3. Fish (Bouts 1 and 3 at WLOU only)

4.5.2 Other Biology Sampling

- Surface water microbes – 1st water chemistry bout of each month (likely Tuesday)
- The riparian habitat assessment can be scheduled anytime within the peak greenness window
- Rapid habitat assessment should be completed between Bouts 1 and 2 if flow conditions allow

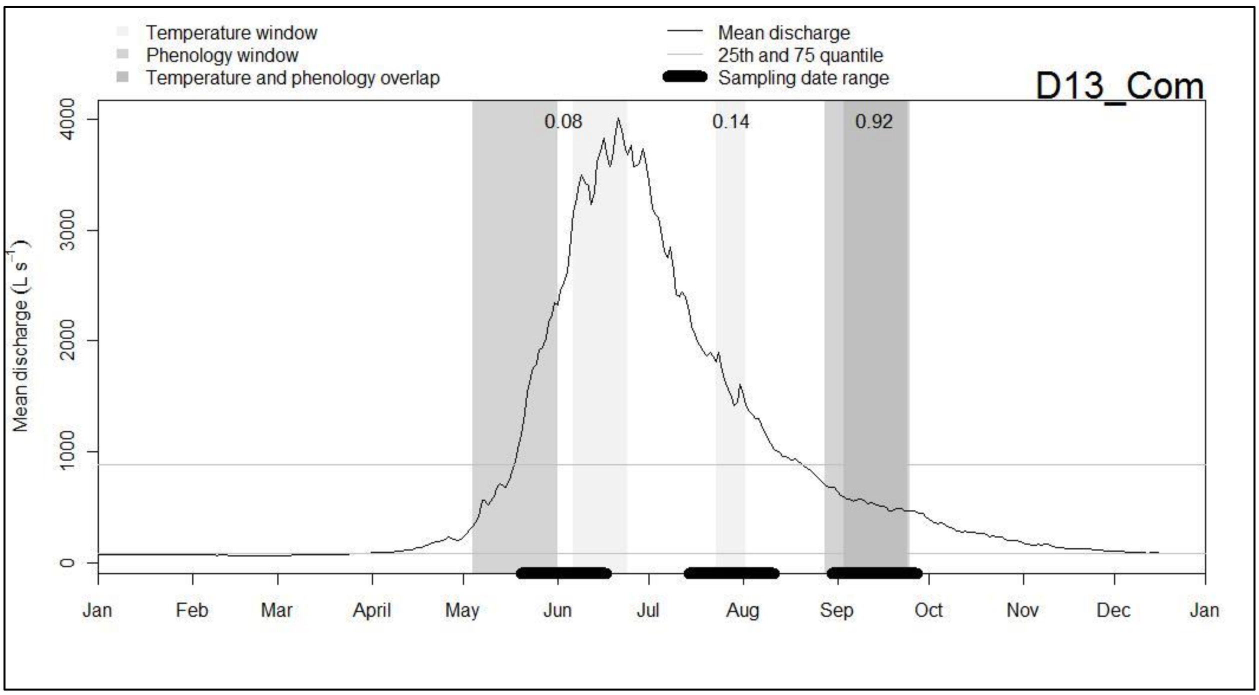


Figure 5. Proposed bouts for biological sampling at Como Creek. Sediment Chemistry occurs during Bouts 1 and 3. No fish sampling at COMO.

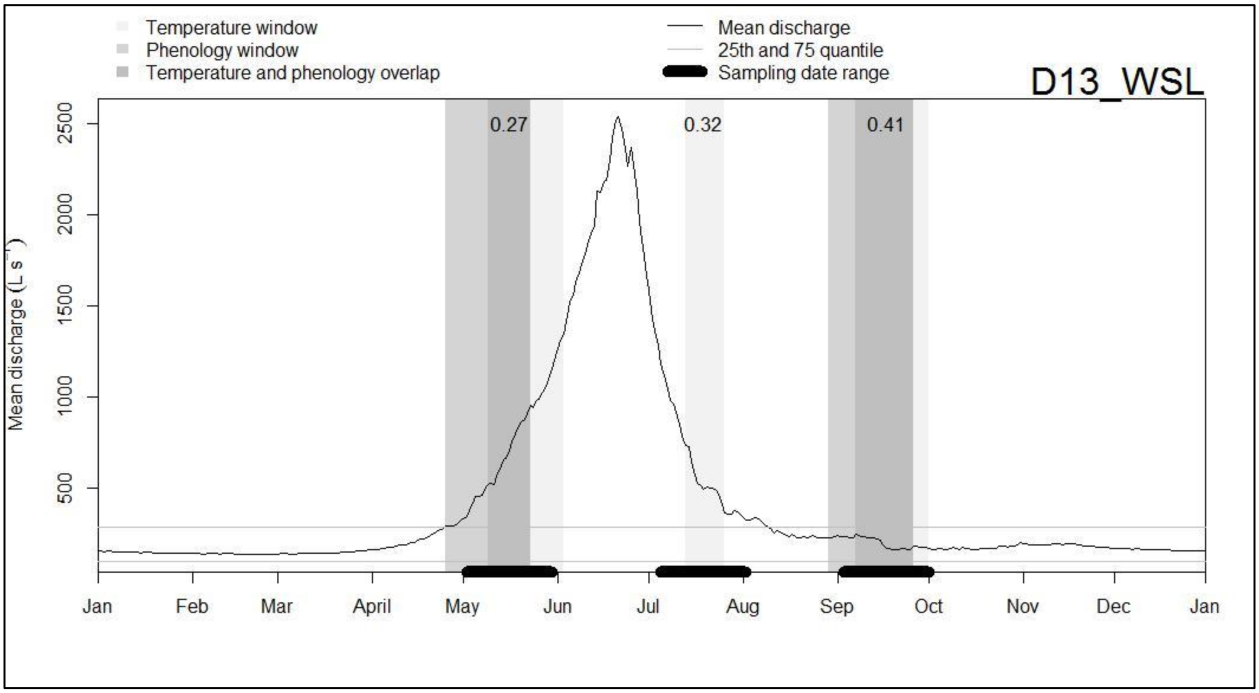


Figure 6. Proposed bouts for biological sampling at West St. Louis Creek. Sediment Chemistry and fish sampling occur during Bouts 1 and 3.

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 streams. Impact level: high (4), medium/high (3), medium/low (2), low (1), none (0). The morphology reach is the entire permitted reach (usually 1 km long). The sensor reach is the length of stream between S1 and S2.

Sample	Requirements	Impact Level	Disturbance
Sensor maintenance	None	0	Wading near sensor sets
Discharge	None	1	Wading only at *established cross-sections*
Reaeration	2 days - no major substrate disturbance, 1 hour with no disturbance to stream	0	Salt/SF6 addition to *sensor reach*
Stream morphology	None	4	Wading through and displacing rocks in *morphology reach* (H)
Aquatic plants	5 days- no scouring or trampling of substrate; low turbidity during sampling	3	Wading and collection at established cross-section transects
Invertebrates	5 days- no scouring or trampling of substrate	3	Wading and disturbance of substrate in *morphology reach*
Periphyton, seston, and benthic microbes	2 weeks- no scouring or trampling of substrate	2	Wading and substrate disturbance in *morphology reach*
Sediment chemistry	1 week- no physical disturbance to depositional zones	2	Wading and substrate disturbance in *morphology reach*
Fish	6 hours- no disturbance that causes turbid conditions	4	Wading extensively throughout *morphology reach*
Surface water chemistry, dissolved gas, isotopes, and surface microbes	Allow stream to clear before sampling minutes, no wading upstream	1	Wading *above S2*
Groundwater chemistry	None	0	Groundwater Removal
Riparian habitat assessment	None	2	Wading and substrate disturbance in *morphology reach*
Rapid habitat assessment	None	2	Wading and substrate disturbance in *morphology reach*

6 SPATIAL SAMPLING STRATEGY

6.1 General Site Sampling Locations

The stream sampling reach is ideally 1000 (± 50) m long. Locations provided in the "proposed" columns in Table 9 and Table 10 are estimates. Field personnel should measure the 1000 m reach, taking care to stay within the permitted boundaries of the site and record coordinates. Specific coordinates at each site will be used for the life of the site.

Stream sampling protocols reference several locations within the site, including the sensor locations for the aquatic site (S1, S2). Other locations provided to the field ecologists include the location of the streamside meteorological station and riparian transects. Benthic biological samples may be taken throughout the entire sampling reach, with locations chosen by the field ecologists at the time of sampling (Figure 7). Minimize benthic sampling in the sensor reach if possible (i.e., no more than 3 replicates for any benthic sampling module). See Table 8 for module-specific sampling locations and rule sets, and Figure 7 for the generic site layout.

Table 8. Module-specific sampling locations, wadeable streams. *May occur at an alternate location if necessary.

Sampling module	Location
Surface water chemistry*	S2 and groundwater wells
Dissolved gas*	S2
Isotopes*	S2 and groundwater wells
Surface water microbes*	S2
Seston*	S2
Discharge	Location chosen by field ecologists/HQ, return to transect unless morphology or flow changes significantly
Reaeration	Injection site upstream of S1, 4 sampling stations spread evenly between S1 and S2
Riparian habitat assessment	Transects determined by HQ and provided to domain staff
Benthic microbes	Same location as periphyton samples
Periphyton	Locations determined by field ecologists within habitat types determined by morphology map; locations may vary from bout to bout
Aquatic plants, bryophytes, lichens, and macroalgae	Transects determined on first sampling bout by field ecologists; return to transects on subsequent bouts
Macroinvertebrates	Locations determined by field ecologists within habitat types determined by morphology map; locations may vary from bout to bout
Fish	Full 1 km permitted reach. Reaches determined on first sampling bout by field ecologists; return to reaches on subsequent bouts
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
Rapid habitat assessment and Stream morphology	From BOT to TOP marker (1 km sampling reach)

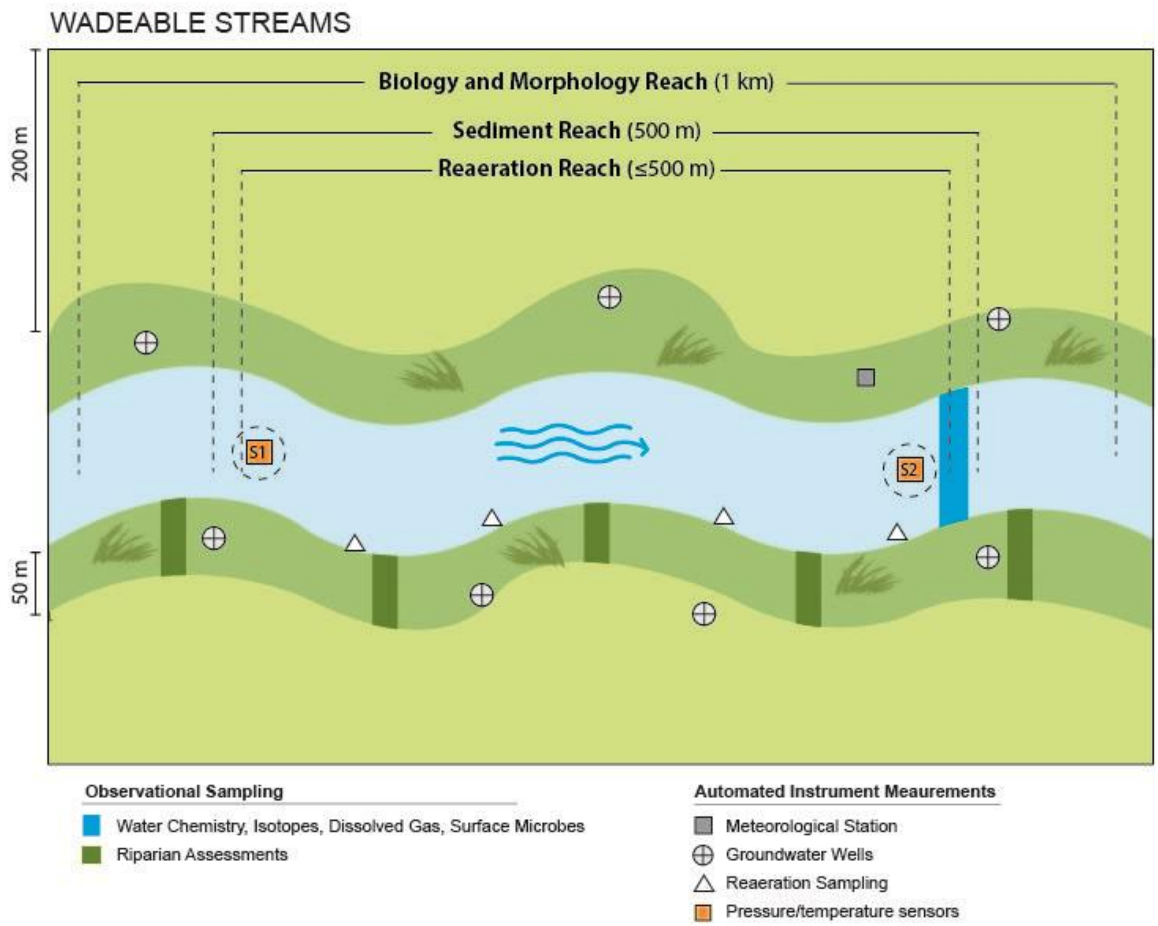


Figure 7. Generic diagram for an AQU site showing sampling locations in a wadeable stream system. Note: the sensor locations are not typically installed as depicted; sediment stations 1 and 2 are divided by the biological reach center not the midpoint between sensors.

Table 9. COMO 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*	40.032198	-105.541301	40.033634	-105.541369
02 - Riparian	Riparian coordinates*	40.033028	-105.541738	40.034229	-105.542038
03 - Riparian	Riparian coordinates*	40.033738	-105.542443	40.03476	-105.542836
04 - Riparian	Riparian coordinates*	40.034483	-105.543094	40.034907	-105.543888
05 - Riparian	Riparian coordinates*	40.034977	-105.544032	40.035004	-105.545031
06 - Riparian	Riparian coordinates*	40.035128	-105.545162	40.034651	-105.545984
07 - Riparian	Riparian coordinates*	40.034982	-105.546314	40.034682	-105.547256
08 - Riparian	Riparian coordinates*	40.034732	-105.547440	40.034663	-105.547748
09 - Riparian	Riparian coordinates*	40.034583	-105.548589	40.034565	-105.548393
10 - Riparian	Riparian coordinates*	40.034554	-105.549760	40.034544	-105.549519
permitted top	Top of permitted reach**	40.03457	-105.5506		
permitted bottom	Bottom of permitted reach**	40.03183	-105.54096		
TOP	Top of sampling reach***	40.034565	-105.550346	40.03458	-105.550275
BOT	Bottom of sampling reach***	40.03183	-105.54096	40.032033	-105.540411
S1	S1 sensor location from SCR	40.034940	-105.545333		
S2	S2 sensor location from SCR	40.034965	-105.54438		
Discharge	Discharge location, decided by Field Science			40.034980	-105.544100

* Riparian coordinates should be approximately evenly spaced throughout the sampling reach, approximately every 100 m starting 50 m from the top and bottom of the sampling reach.
**Do not sample outside of this boundary
***Should be 1000 m unless permitting restricted

Table 10. WLOU 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*	39.890685	-105.910338	39.890684	-105.910661
02 - Riparian	Riparian coordinates*	39.890802	-105.911495	39.890730	-105.911630
03 - Riparian	Riparian coordinates*	39.891063	-105.912615	39.891013	-105.912709
04 - Riparian	Riparian coordinates*	39.891211	-105.913769	39.891155	-105.913623
05 - Riparian	Riparian coordinates*	39.891322	-105.914931	39.891235	-105.914574
06 - Riparian	Riparian coordinates*	39.891409	-105.916093	39.891353	-105.915284
07 - Riparian	Riparian coordinates*	39.891356	-105.917261	39.891385	-105.916305
08 - Riparian	Riparian coordinates*	39.891256	-105.918424	39.891463	-105.917238
09 - Riparian	Riparian coordinates*	39.891080	-105.919570	39.891218	-105.918279
10 - Riparian	Riparian coordinates*	39.890812	-105.920682	39.891122	-105.919023
permitted top	Top of permitted reach**	39.89062	-105.921211		
permitted bottom	Bottom of permitted reach**	39.890698	-105.909739		
TOP	Top of sampling reach***	39.89062	-105.921211	39.890796	-105.919374
BOT	Bottom of sampling reach***	39.890698	-105.909739	39.890684	-105.91024
S1	S1 sensor location from SCR	39.891330	-105.914420		
S2	S2 sensor location from SCR	39.89068	-105.91133		
Discharge	Discharge location, decided by Field Science			39.890649	-105.911196

* Riparian coordinates should be approximately evenly spaced throughout the sampling reach, approximately every 100 m starting 50 m from the top and bottom of 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 to minimize local disturbance over the life of the project for locations that are accessed frequently (e.g., sensors; Figure 8, Figure 9). Field ecologists must use established paths and access points when possible to avoid causing disturbance to the site.

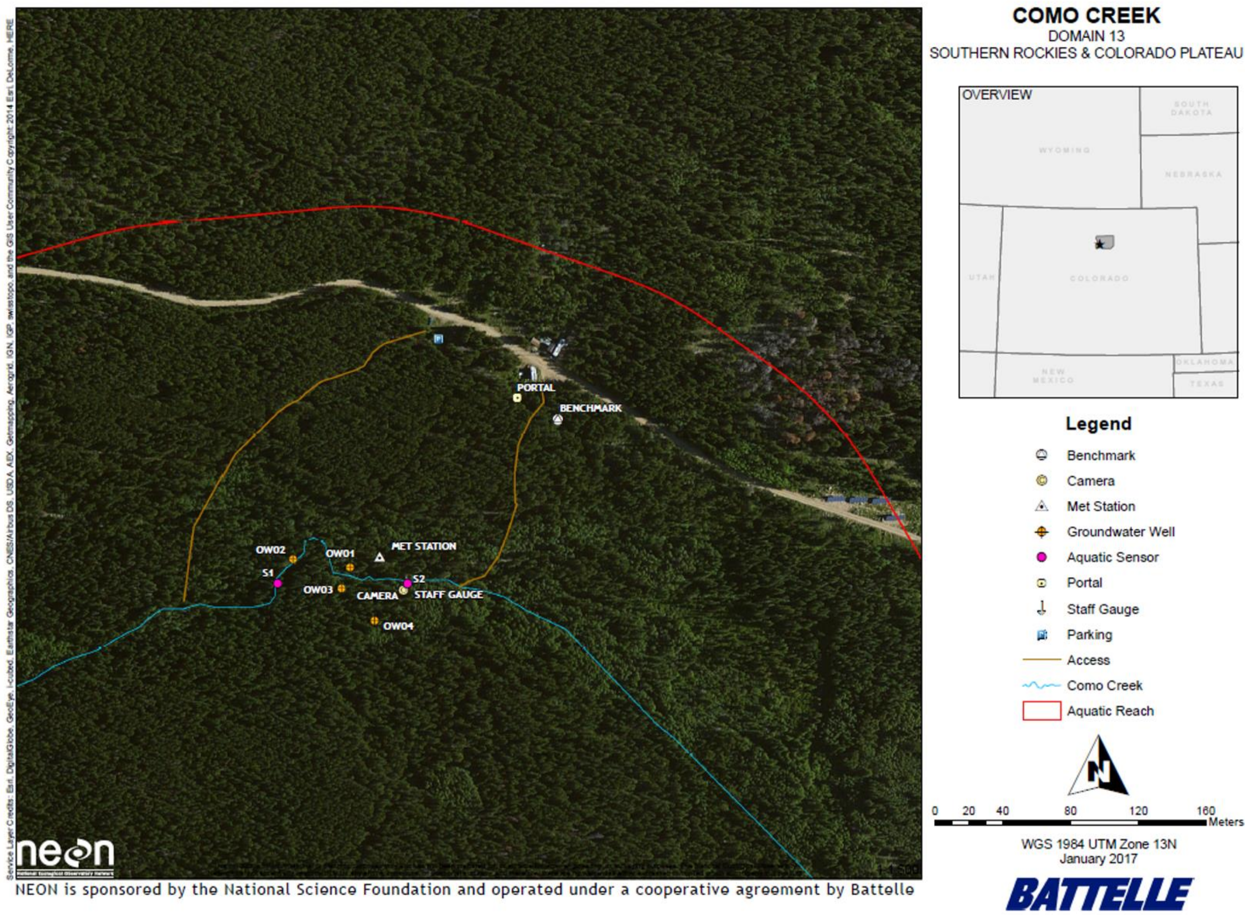


Figure 8. Site access and instrument locations at D13 Como Creek. An additional path (not depicted) is used to access the bottom of the sampling reach.

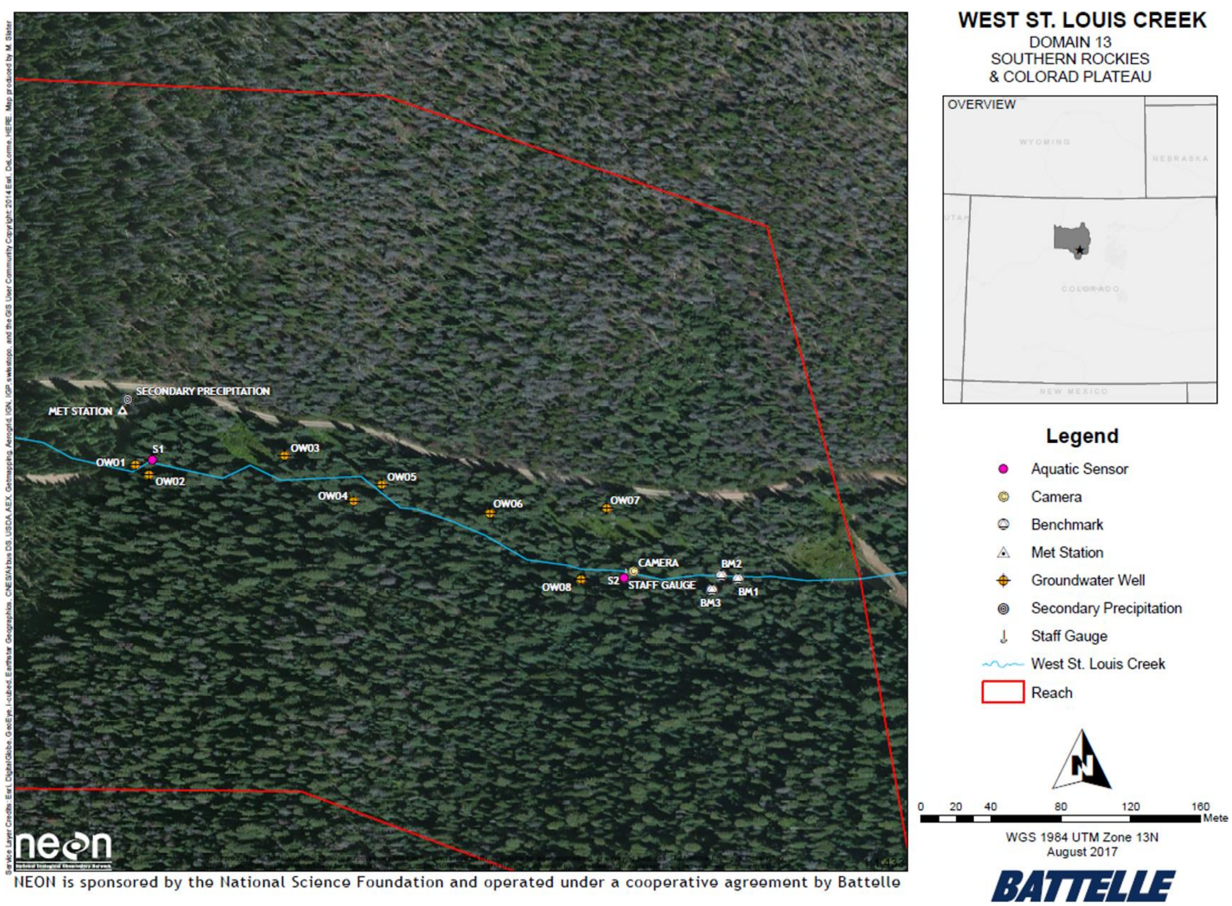


Figure 9. Site access and instrument locations at D13 West St. Louis Creek.

6.3 Riparian Sampling Locations

Riparian coordinates are determined prior to sampling at HQ. Coordinates 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. Riparian transects are numbered from 1-10 starting at the downstream end of the sampling reach (Figure 10, Figure 11).

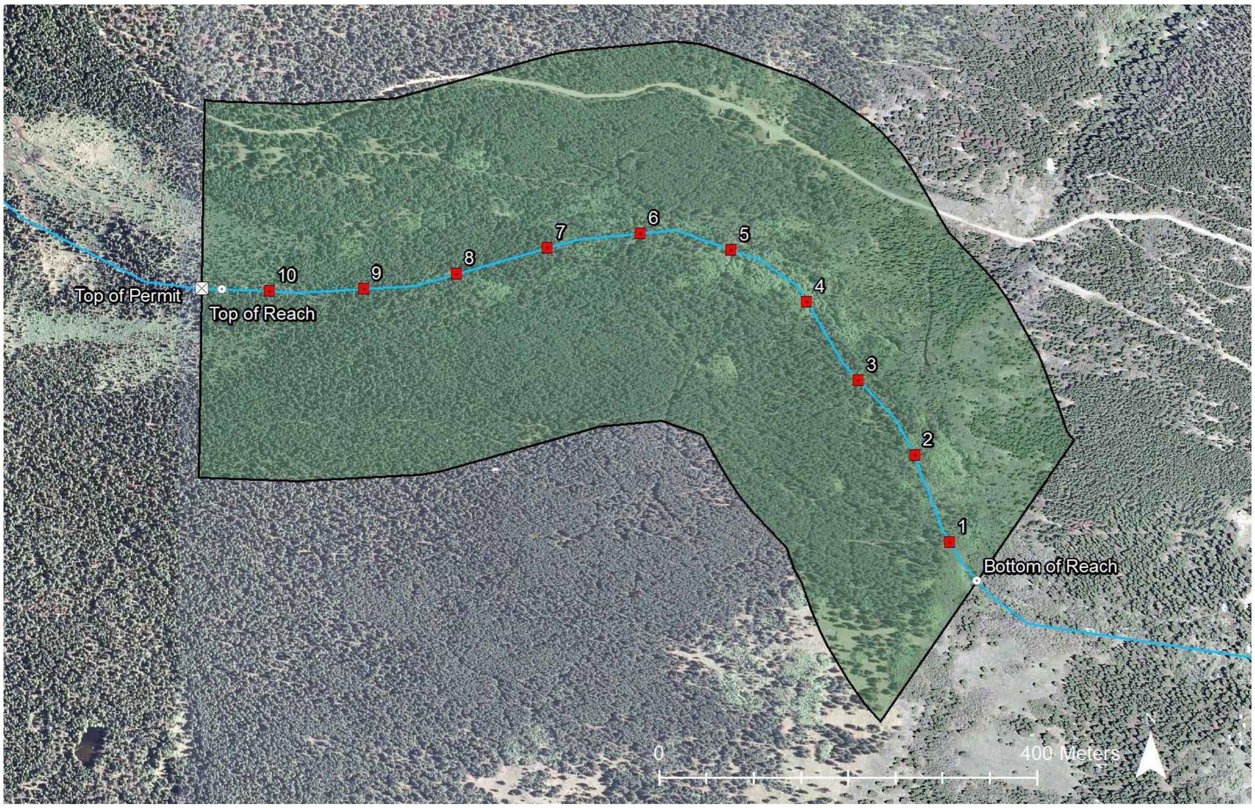


Figure 10. COMO ideal riparian sampling design

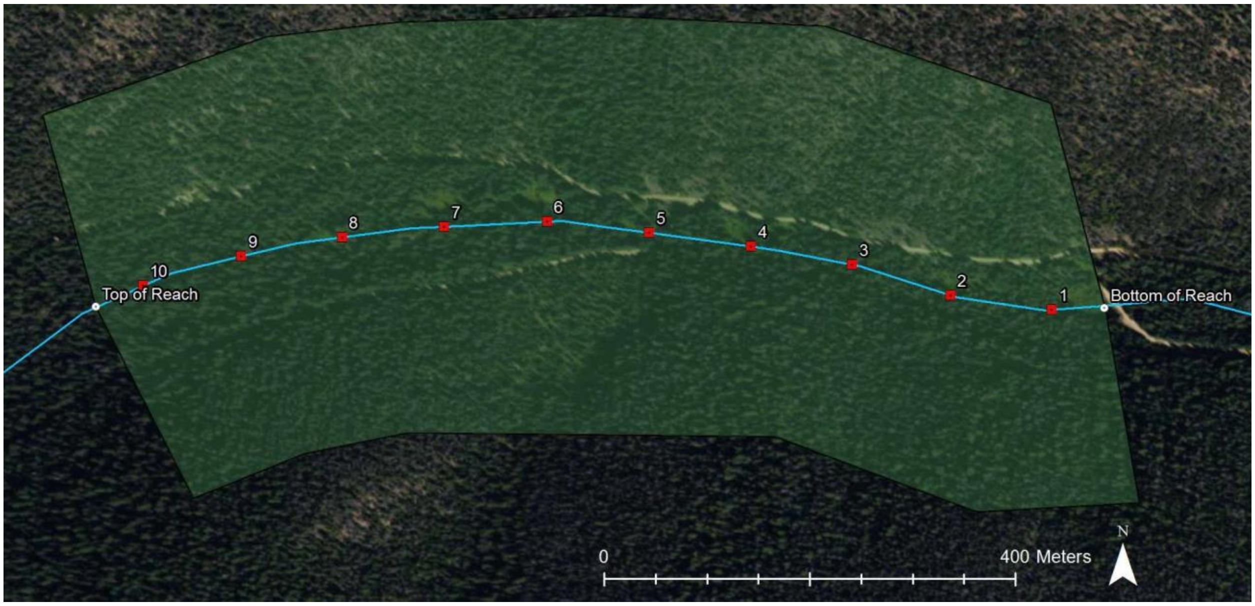


Figure 11. WLOU Ideal Sampling Design

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.

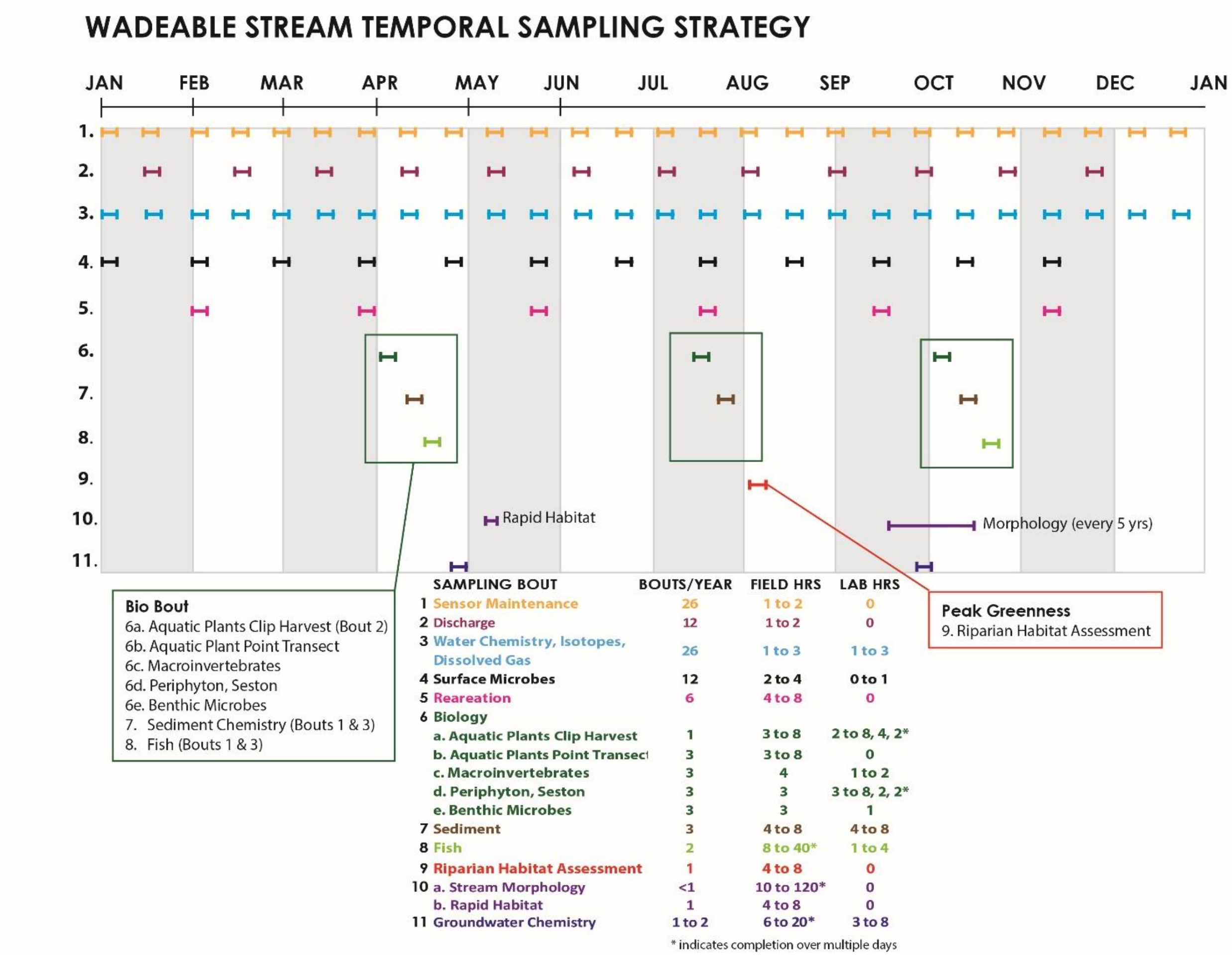
Hargrove, W. W. and J. Pickering. 1992. Pseudoreplication: a sine qua non for regional ecology. Landscape Ecology 6: 251-258.

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.

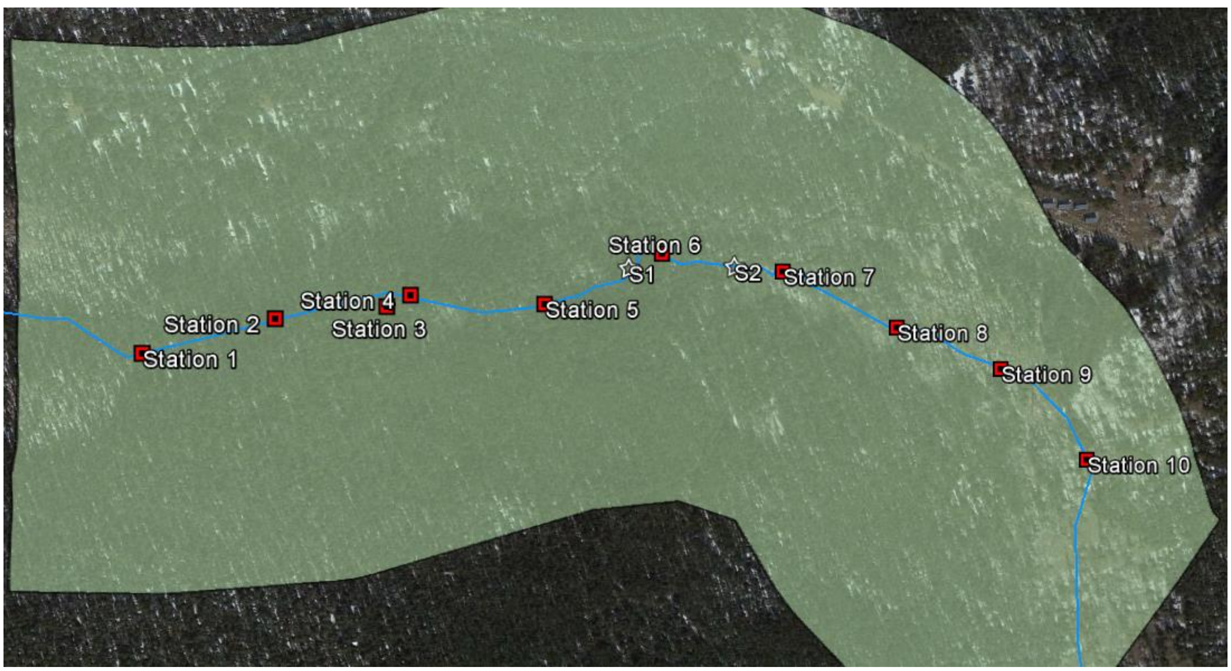
APPENDIX A WADEABLE STREAMS



APPENDIX B OBSOLETE LOCATIONS, COMO

Groundwater chemistry samples were initially collected at wells both near and far from the instream sensor sets, and have since been narrowed to 4 wells based on the strategy described above.

COMO Obsolete Locations		
Station	Latitude	Longitude
1	40.034344°	-105.549536°
2	40.034577°	-105.548397°
3	40.034665°	-105.547445°
4	40.034743°	-105.547242°
5	40.034693°	-105.546073°
6	40.035048°	-105.545029°
7	40.034941°	-105.543929°
8	40.034556°	-105.542876°
9	40.034268°	-105.541902°
10	40.033628°	-105.541101°
S1	40.034940	-105.545333
S2	40.034965	-105.544380
Discharge	40.034980	-105.544100
TOP of Reach	40.034570	-105.550600
BOT of Reach	40.031830	-105.540960



APPENDIX C OBSOLETE LOCATIONS, WLOU

Groundwater chemistry samples were initially collected at wells both near and far from the instream sensor sets, and have since been narrowed to 4 wells based on the strategy described above.

WLOU Obsolete Locations		
Station	Latitude	Longitude
1	39.890954	-105.920064
2	39.891209	-105.918991
3	39.891345	-105.917923
4	39.891395	-105.916838
5	39.89137	-105.91579
6	39.891365	-105.914733
7	39.891286	-105.913687
8	39.891094	-105.912674
9	39.890837	-105.911784
10	39.890657	-105.910698
S1	39.89133	-105.91442
S2	39.89068	-105.91133
Discharge		
TOP of Reach	39.890529	-105.920993
BOT of Reach	39.890698	-105.909739